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Inhibition of thromboxane biosynthesis by 3-pyridinol carboxypentyl ethers substituted with a hydroxylated octyl chain

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Abstract—Racemic 6-[4-(3′-hydroxy-1′-octenyl)-3-pyridyloxy] hexanoic and 6-[4-(3′-hydroxyoctyl)-3-pyridyloxy] exanoic acids have been synthesized and their activity as inhibitors of the biosynthesis of thromboxane A_2 in human serum has been studied, in comparison with isomers having the eight-carbon chain in the 2 position. Very high, selective activity was found for the new 4-substituted 3-pyridinol ethers, whereas the 2-substituted compounds showed no action.

Pyridines substituted in position 3 with a carboxylic acid side chain are inhibitors of thromboxane A_2 (TxA_2) synthase; the side chain can include an aryl (Tanouchi et al 1981) or a benzofuryl (Johnson et al 1986) group. The optimum distance between heterocyclic nitrogen and carboxy group is 8·5–10 Å. For further substituted pyridines the 4-position should be preferred since it has been previously shown that the pyridine nitrogen must be sterically unhindered for activity (Akahane et al 1984). The pyridine derivatives studied by Corey et al (1983), showing the two prostaglandin (PG) chains in 3- and 4-positions, inhibited TxA_2-synthase with an IC50 of about 1 μM.

In a direct comparison between isomeric 2,3- and 3,4-disubstituted pyridines, where the 3-position was occupied by the ω-carboxypentyloxy group and the other position (2 or 4) by the carboxaldehyde guanylhydrazone residue, we found that both were active inhibitors of human TxA_2-synthase, with an IC50 of 2 μM for the 2-substituted derivative and 0·2 μM for the 4-substituted one. The first was a more selective inhibitor, lowering PGE_2 only at a concentration of 100 μM, while the second completely inhibited PGE_2 production at 10 μM (Desideri et al 1986).

It was therefore interesting to compare another pair of disubstituted isomers. Desideri et al (1985) described the synthesis of some lipophilic ethers of 2-substituted 3-pyridinol and their hypolipidaemic activity in rats. Among them 6-[2-(3′-hydroxy-1′-octenyl)-3-pyridyloxy]hexanoic acid (1) and 6-[2-(3-hydroxyoctyl)-3-pyridyloxy]hexanoic acid (2) were particularly interesting as heterocyclic analogues of 7-oxa-prostaglandins.

We planned to synthesize their isomers 3 and 4, showing the alkyl chain in the 4-position, and to investigate the two pairs of compounds as possible TxA_2-synthase inhibitors, using serum TxB_2 production as an index of platelets TxA_2-synthase activity.

Materials and methods

Compounds 1 and 2 have already been obtained by us; the synthesis of compounds 3 and 4 in racemic form, through the esters 6 and 7, was accomplished by an analogous method (Fig. 1) (Desideri et al 1985).

The yields and the physical properties of the new compounds are as follows. Ethyl 6-[4-(3′-oxo-1′-*trans*-octenyl)-3-pyridyloxy]hexanoate (6), yield 85%, colourless oil. IR(film): cm^{-1} 1730(C=O est.), 1710(C=O ket.). NMR ($CDCl_3$): δ ppm 8·35(s,1H,H_2); 8·20(d,1H,H_6, J=6 Hz); 7·70(d,1H,PyCH=C, J=16 Hz); 7·35 (d,1H,H_5, J=6 Hz); 6·90 (d,1H,C=CHCO, J=16 Hz); 4·40–3·90 (m, 4H,OCH_2,$COOCH_2$); 2·60 (t,2H,$COCH_2$); 2·30 (t,2H,CH_2COO); 2·00–1·05 (m, 15H,6CH_2, CH_3); 0·90 (t,3H,CH_3). Ethyl 6-[4-(3′-hydroxy-1′-*trans*-octenyl)-3-pyridyloxy]hexanoate(7), yield 56%, colourless oil. IR(film): cm^{-1} 3500–3100(OH), 1740(C=O). 6-[4-(3′-Hydroxy-1′-*trans*-octenyl)-3-pyridyloxy]hexanoic acid(3), yield 76%, m.p. 79–81°C. IR(KBr): cm^{-1} 3550–2400(OH), 1730(C=O). NMR($CDCl_3$): δppm 8·85–8·50(b.s., 2H,2OH); 8·30(s,1H,H_2); 8·20(d,1H,H_6, J=4 Hz); 7·35(d,1H,H_5, J=4 Hz); 6·9(d,1H,PyCH=C, J=16 Hz); 6·55(dd,1H,C=CHCO, J_{AB}=16 Hz, J_{BX}=6 Hz); 4·35(m,1H,CH, J_{BX}=6 Hz); 4·10(t,2H,OCH_2); 2·35(t,2H,CH_2COO); 2·00–1·10(m,14H,7CH_2); 0·85(t,3H,CH_3). 6-[4-(3′-Hydroxyoctyl)-3-pyridyloxy]hexanoic acid(4), yield 60%, m.p. 100–105°C. IR(KBr): cm^{-1} 3600–3200(OH), 2700–2200 (ac. OH), 1710(C=O). NMR($CDCl_3$): δ ppm 8·20(m,2H,H_2,H_6); 7·10(d,1H,H_5); 6·55–5·95 (b.s., 2H,2OH); 4·00(t,2H,OCH_2); 3·65 (m,1H,CH); 2·70(t,2H,$PyCH_2$); 2·25(t,2H,CH_2COO), 2·00–1·05(m,16H,8CH_2); 0·85(t,3H,CH_3).

Venous blood from healthy volunteers was collected in glass tubes without anticoagulant, immediately mixed with different

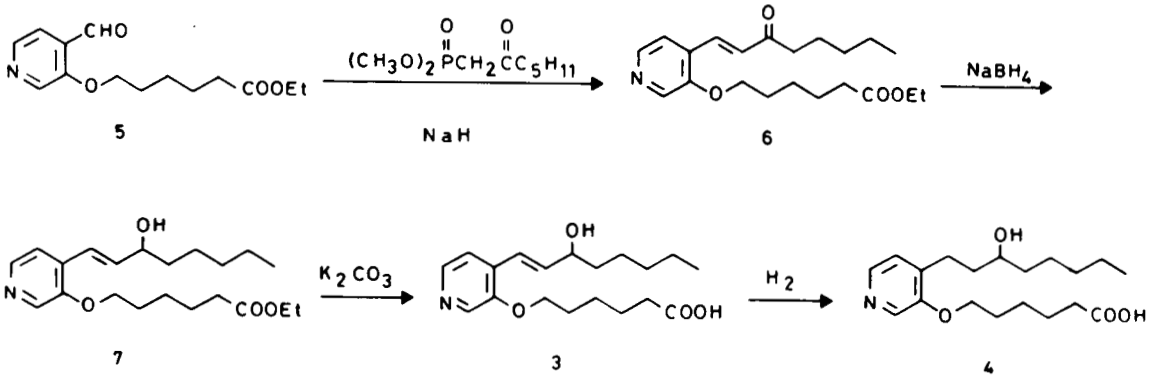

FIG. 1. Synthesis of compounds 3 and 4.

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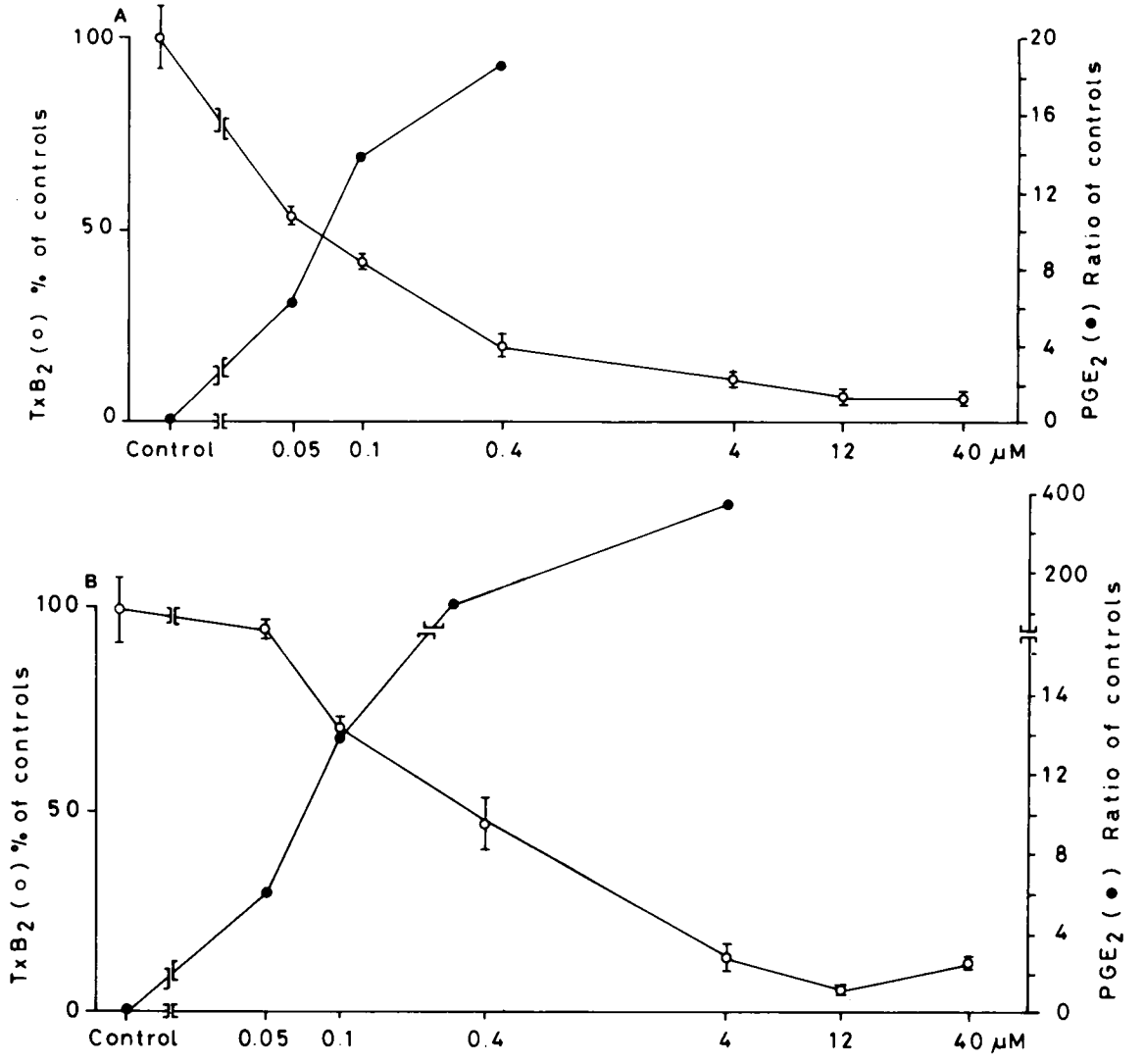

FIG. 2. Effect of A, compound 3 and B, compound 4 on human serum TxB_2 and PGE_2 generation. The compounds were incubated with native blood for 1 h at 37°C. Control values of TxB_2 and PGE_2 were 436 ± 36 and $1{\cdot}70 \pm 0{\cdot}16$ ng mL^{-1}, respectively (means $\pm$ s.e.m.; n = 6).

amounts of the compounds to be tested or their solvent (phosphate buffer, pH 7·4) and incubated at 37°C for 1 h. The serum was separated by centrifugation and stored at −20°C until assayed for TxB_2 and PEG_2. The two prostanoids were quantitated by specific radioimmunoassays as previously described (Desideri et al 1986; Bertelé et al 1984). Cross reactivity of PEG_2 antiserum with compounds 3 and 4 was lower than 0·03 and 0·01%, respectively.

Results and discussion

No significant reduction of serum TxB_2 occurred in blood incubated in the presence of compounds 1 and 2 up to 100 μM concentration (data not shown). Compounds 3 and 4 significantly reduced serum TxB_2, reaching maximal effect (more than 95% inhibition) at 12 μM concentration (Fig. 2). The IC50s calculated from the dose-response plots were 0·06 and 0·38 μM for compounds 3 and 4, respectively. Concomitantly with TxB_2 synthesis inhibition, serum PGE_2 rose (18 and 300 times control values for the two compounds). This suggests that 3 and 4 act as selective inhibitors of TxA_2 synthesis, since the cyclic endoperoxides spared by TxA_2-synthase inhibitors are diverted to the synthesis of other prostaglandins (Bertelé et al 1984).

In accordance with these results and previous experience (Akahane et al 1984), 2-alkyl-substituted pyridines are not active as inhibitors of TxA_2-synthase, unless they are substituted in a side chain with a basic group as in the case of guanylhydrazones (Desideri et al 1986); the hypothesis that this group, rather than the pyridine nitrogen, can interact with a proton donor on the active site of the enzyme should be demonstrated with other examples.

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Action of mefloquine on toad isolated rectus abdominis muscle

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Abstract—The effects of mefloquine and quinine on acetylcholine-induced contractures of the toad rectus abdominis muscle have been investigated. Both drugs depressed acetylcholine-induced contractures in a dose-related and non-competitive manner. A partial reversal of the block was observed in the presence of 4-aminopyridine (10, 50 μM) and increased Ca^{2+} content (1·25 ×) of the Ringer solution. In both cases, the mefloquine-induced blockade was more readily reversed than that induced by quinine. Mefloquine and quinine at concentrations greater than 100 and 500 μM, respectively, also elicited a direct contractile response on the muscle. Quantitative differences in their contractile activity have been attributed to the greater lipid solubility and tissue binding affinity of mefloquine.

Mefloquine, a 4-quinolinemethanol derivative structurally related to quinine, is a highly effective antimalarial agent which has been found to be active against chloroquine-resistant strains of *Plasmodium falciparum* (Doberstyn et al 1979). Quinine has been shown to interfere with the contractile activity of skeletal muscle (Grewal & Sharma 1960; Isaacson et al 1970). Whether mefloquine affects muscle in the same way is not known, although it has been noted to cause muscle weakness during antimalarial therapy (Hall et al 1977). We have made a comparative study on the actions of mefloquine and quinine on the toad isolated rectus abdominis muscle. As to be expected from their chemical identity, both drugs affect the muscle in a similar way but quantitative differences are observed.

Methods

The toad (*Bufo melanostictus*) isolated rectus abdominis muscle was mounted in a bath containing Ringer solution of the following composition (mM): NaCl 111, KCl 1·9, $CaCl_2$ 1·4, NaH_2PO_4 0·083, $NaHCO_3$ 2·4, and glucose 0·55. The tissue was aerated with 5% carbon dioxide in oxygen at room temperature (28°C) and equilibrated for 45 min before being challenged with drugs.

The cumulative dose-response curves for acetylcholine added at 90 s intervals were obtained in the absence or presence of mefloquine or quinine added after a 30 min rest. After a further 30 min rest, a final dose-response curve was obtained. No more than three cumulative dose-response curves were obtained with each preparation. Three consecutive control cumulative dose-response curves were also obtained to assess the viability of the tissue. For construction of the cumulative log concentration-response curves, all responses were expressed as a percentage of the maximum height of the control curve.

To assess the effect of increased Ca^{2+}, the Ca^{2+} content of the Ringer solution was increased 1·25× to 1·8 mM and the cumulative curves were obtained as before in the presence of mefloquine (10 μM) or quinine (50 μM). Three consecutive control cumulative dose-response curves were also obtained.

To assess the effect of 4-aminopyridine, the tissue was exposed to the drug (10 or 50 μM) for 10 min after which the cumulative dose-response curve was obtained. After a 30 min rest, 4-aminopyridine and inhibitor were added to the bath and the cumulative dose-response curve repeated. A final curve was obtained 30 min later in the presence of 4-aminopyridine alone. Controls were three successive cumulative dose-response curves obtained in the presence of 4-aminopyridine, with the usual 30 min rest between determinations.

To assess the contractile activity of mefloquine and quinine on the muscle, their cumulative dose response curves were obtained using a 90 s contact time between each addition. In both cases, the maximum response of the muscle could not be recorded because of the limited aqueous solubility of the inhibitors. Thus, the responses were expressed as a percentage of the height attained with the highest concentration of the inhibitor that could be obtained in solution. Two more cumulative dose-responses were repeated subsequently on the same tissue with the usual 30 min rest period to assess muscle responsiveness.

Solutions of erythro-DL-mefloquine hydrochloride (Roche Pharmaceuticals), quinine sulphate BP (Pharmaceutical Department, Republic of Singapore), 4-aminopyridine (98%, Aldrich Chemical Company) and acetylcholine iodide (Sigma Chemical Company) were prepared in distilled water. Appropriate dilutions were made from these solutions. All other chemicals were of analytical grade.

Results

The cumulative log concentration-response curves for acetylcholine in the absence and presence of mefloquine (1, 10, 100, 300 μM) and quinine (10, 50, 100, 1000 μM) showed that both drugs depressed the acetylcholine-induced contractures in a dose related manner and caused the curves to be displaced to the right in a non-parallel manner. That the observed decrease in the maximal response was due to an effect of mefloquine and quinine was validated by a comparison with the three control curves obtained on the same tissue in the absence of inhibitors. These control experiments showed a corresponding decrease of 11·3% (s.e.m. 2·6%) and 14·4% (s.e.m. 2·6%) in the maximal responses of the 2nd and 3rd curves, respectively (n = 6–13 experiments).

An anomaly was noted in the cumulative log concentration-response curves obtained in the presence of mefloquine (100, 300 μM) and quinine (1000 μM) in that the % maximal responses elicited at low acetylcholine concentrations (< 500 μM) were higher than those observed at lower inhibitor concentrations,

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