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IMPERIALINE ESTERS AND THEIR M_2-CHOLINE-BLOCKING ACTIVITY

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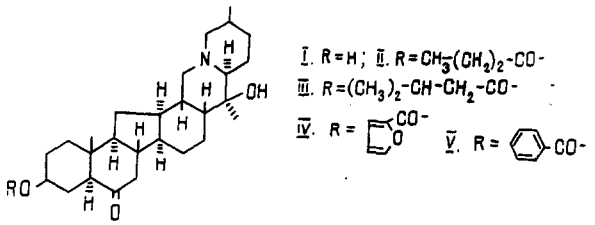
Four new imperialine esters, with butyric, isovaleric, pyromucic, and benzoic acids, have been synthesized, and their M_2-choline-blocking activity has been studied.

Cardioselective, i.e., M_2-choline-blocking, properties have been detected previously in imperialine (I) — the main alkaloid of the plant *Petilium* [1, 2]. At the present time it has been established that M_4-choline-receptors predominate in smooth-muscle organs and M_3-choline-receptors in secretory organs [3]. With aim of finding more active compounds and revealing their tropicity to various subtypes of M-receptors, from imperialine (I) [4-6] we have synthesized a number of its derivatives: butyrylimperialine (II), isovalerylimperialine (III), furoylimperialine (IV), and benzoylimperialine (V) by its reaction with the corresponding acid chlorides.



In compounds (II-V) the acyl group was present exclusively at C-3, since the tertiary hydroxy group at C-20 in the imperialine molecule does not undergo acylation under the conditions that we describe. All the compopunds are new, and their structures have been confirmed by mass spectrometry.

It can be seen from Table 1 that, with respect to their M-choline-blocking activities, butyryl- and isovalerylimperialines are 3 times more effective than imperialine and almost reach the activity of atropine. Pyromucyl- and benzoylimperialines are more than 5 times superior to imperialine and, on the whole, are superior to atropine. As can be seen from the last column of the table, on the whole the ratio of the degrees of expression of M-choline-blocking activity of the imperialine derivatives for the organs (heart, intestine, and salivary gland) remain the same as for imperialine itself — i.e., the M_2-blocking activity is most pronounced for the heart, is 1-2 orders of magnitude less pronounced on the M_4 subtype of receptors of the intestine, and is least for the M_3 subtype of the salivary gland.

EXPERIMENTAL

The individuality of the substances was checked by TLC on KSK silica gel (100 nm)—gypsum (9:1) plates in the chloroform−methanol (9:1) system. The revealing agent was iodine vapor. Mass spectra were taken on a MKh-1310 instrument.

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TABLE 1. Some Chemical Constants, Acute-Toxicity Indices, and M-Choline-Blocking Activities of Imperialine and Some of its Derivatives on the Heart, Intestine, and Salivary Gland in Comparison with Atropine

Alkaloid	Mol. mass	LD_{50}, μmole/kg	Heart		Intestine		Salivary gland		Ratio of M-blocking activities on the heart:intestine: salivary gland
			ED_{50}, μmole/kg	% of the activity of atropine	ED_{50}, μmole/kg	% of the activity of atropine	ED_{50}, μmole/kg	% of the activity of atropine	
Atropine sulfate	387	191±48	0,031±0,012	100	0,0034±0,0013	100	0,016±0,004	100	0,5:0,5:1
Imperaline (HCl, I)	465	129±36	0,124±0,044	25,0	0,52±0,18	0,65	26,2±11,4	0,061	410:11:1
Butyrylimperialine (HCl, II)	499	92±19	0,042±0,014	73,8	0,22±0,09	1,54	13,6±4,7	0,12	615:13:1
Isovalerylimperialine (HCl, III)	513	82±16	0,035±0,016	88,6	0,27±0,12	1,26	16,0±4,6	0,1	886:13:1
Furoylimperialine (HCl, IV)	523	98±22	0,027±0,011	114,8	0,054±0,018	6,3	10,3±3,1	0,16	717:39:1
Benzoylimperialine (HCl, V)	533	92±24	0,021±0,008	147,6	0,041±0,017	8,3	15,6±6,3	0,1	1476:83:1

Butyrylimperialine (II). A mixture of imperialine (0.7 g), pyridine (2 ml), and butyryl chloride (1 ml) was left at room temperature for 24 h, and then the solvent was driven off in vacuum. The residue was dissolved in 5% sulfuric acid, and the solution was washed with ether and was then made alkaline with ammonia and extracted with chloroform. The residue after the chloroform had been distilled off was chromatographed on a column of silica gel, and from a chloroform eluate we isolated butyrylimperialine (0.4 g) with mp 153-154°C (acetone), M^+ 499.

Isovalerylimperialine (III). By a procedure similar to that for butyrylimperialine (II), imperialine (0.7 g) and isovaleryl chloride (1 ml) yielded amorphous isovalerylimperialine, M^+ 513 (0.25 g).

Furoylimperialine (IV). A mixture of pyromucic acid (0.7 g) and thionyl chloride (5 ml) was heated for 3 h. Then the excess of thionyl chloride was distilled off, and, with cooling, 3 ml of pyridine and 1 g of imperialine (I) were added to the oily residue, and the mixture was left at room temperature for three days. After elimination of the pyridine, the residue was dissolved in 5% sulfuric acid, and the solution was made alkaline with ammonia and extracted with ether. When the ethereal solution was concentrated, crystals of imperialine (0.05 g) deposited. From the imperialine mother liquor we then isolated 0.12 g of furoylimperialine with mp 172-174°C (ether), M^+ 523.

Benzoylimperialine (V). A mixture of imperialine (1.07 g), pyridine (5 ml), and benzoyl chloride was left for 24 h. Then the pyridine was distilled off in vacuum. The residue was dissolved in 5% sulfuric acid, and the solution was made alkaline with ammonia and was extracted with chloroform. The residue after the chloroform had been distilled off was chromatographed on a column of silica gel, and benzene–methanol (3.5:0.5) eluates yielded 1 g of benzoylimperialine (V), with mp 209-212°C (benzene), M^+ 533.

The acute toxicities of the compounds with a determination of LD_{50} values were studied by intravenous injection into male white mice weighting 20-24 g. The M-choline-blocking activities of the compounds on the heart and salivary glands were tested on anesthetized (ethaminal sodium, 50 mg/kg intraperitoneally) male white rates weighing 280-320 g. Stimulation of the M-receptors of the organs concerned was brought about by the intravenous injection of carbachol in the maximum tolerated dose of 0.02 mg/kg. This dose of carbachol caused a reduction in the frequency of cardiac contractions from 348 ± 46 to 84 ± 16 and the secretion of 0.76 ± 0.22 g/kg of saliva.

Experiments on the isolated small intestine of the rat were condcuted by Magnus' method using Tyrode's solution. Stimulation of the M-receptors of the intestine was brought about with carbachol in concentrations of 10^{-7}-$2 \cdot 10^{-7}$ g/ml, causing a close to maximum spasm.

For all three series of experiments we calculated EC_{50} and ED_{50} values graphically from the antagonism of the alkaloids under investigation to the efects of carbachol. To plot a graph we used 3 or 4 doses or concentrations of the substances suppressing the effects of carbachol by less than, and also by more than, 50%. Each dose or concentration of alkaloid was tested in 5-6 experiments, and the mean of the results was calculated. The statistical treatment was carried out by the Facstorp–Pedersen method at $P = 0.05$.

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