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Synthesis of some new neostigmine methyl sulphates and related compounds

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Studies on anticholinesterase agents (anti-ChE) like physostigmine [1] resulted in the discovery of neostigmine methylsulphate, i.e. 3-[[(*N*,*N*-dimethylamino)carbonyl]oxy]-*N*′,*N*′,*N*′-trimethylbenzenaminium methyl sulphate (**1**), which was introduced in therapy in 1931 due to its efficiency against *Myasthenia gravis* [2]. Structure-activity studies indicated the importance of the presence of two alkyl groups at the carbamate nitrogen atom and a substituted amino group at the other part of the molecule [3–5]. Effects of additional substituents, e.g. at the benzene ring in phenyl carbamates, had not yet been fully recognized.

Therefore, the main goal of this work was to synthesize some new neostigmine derivatives **2**–**7**, and three other related compounds **8**–**10** (Scheme), to search for more satisfatory anti-ChE agents. It had been assumed that a substituent in the 4-position would affect activity of the compounds to a greater extent. This assumption can be supported by the observation that anomalously enhanced transmissibility of electronic effects from substituents at the benzene ring to the carbamyl moiety has been observed [6]. However, as the substituent effect for this class of compounds is rather unpredictable [7], a synthetic work seemed to be necessary.

Compounds **1**–**10** were obtained in a reaction of dimethyl sulphate in THF with the corresponding carbamates **11**–**17**, thiocarbamate, thioncarbamate or urea derivative. Compounds **14** and **17** were very unreactive due to the presence of electron-withdrawing groups (chloro and acetyl, respectively) in the *para* position relative to the dimethylamino group. The preparation of compound **4** (R=Cl) required further heating at 40 °C, for 4 h and compound **7** (R=Ac) was obtained on stirring the reaction mixture for 15 days at room temperature. Compounds **1**–**3**, **5**–**6** and **8**–**9** precipitated just on mixing the reagent with the substrate at room temperature. Moreover, all attempts to methylate the 4-nitro-carbamate led to unsuccessful results. The reaction was carried out in THF, acetone and toluene, on heating to reflux by 7 days. In the experiments with other methylating reagents such as MeI and *p*-Me-$C_6H_4SO_3Me$ (both in THF and toluene under reflux) and F_3CSO_3Me in CH_2Cl_2, at room temperature

Scheme

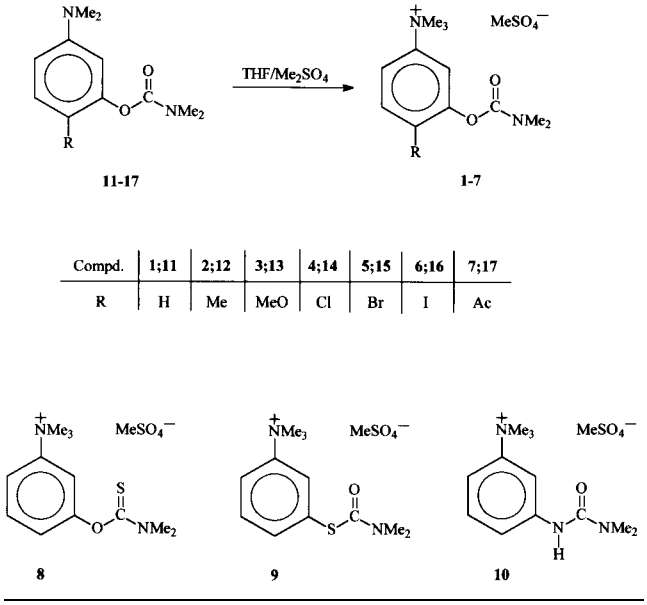

Table: ^{1}H NMR Chemical shifts, coupling constants, physical and IR data, and yields for the neostigmine methyl sulphates and related compounds

Compd.	R	δ/ppm[a]								$J_{i,k}$[b]/Hz		M.p.	ν_{max}[a]	Yields
		N^+Me_3	$MeSO_4^-$	2-H	4-R	5-H	6-H	s-Me[c]	a-Me[c]	$J_{2,6}$	$J_{5,6}$	(°C)	(cm^{-1})	(%)
1	H	3.74	3.66	7.66	7.27	7.58	7.83	2.99	3.12	1.9	8.3	143–5	1725	70
2	Me	3.68	3.64	7.58	2.22	7.42	7.75	3.00	3.15	2.8	8.7	109–11	1718	76
3	OMe	3.71	3.70	7.56	3.87	7.13	7.86	2.99	3.12	3.3	9.4	167–9	1720	85
4	Cl	3.69	3.63	7.83	–	7.64	7.87	2.98	3.14	3.0	9.0	134	1720	60
5	Br	3.74	3.67	7.80	–	7.81	7.85	3.01	3.17	2.9	8.9	139–40	1720	70
6	I	3.72	3.66	7.76	–	8.02	7.71	3.01	3.19	2.9	8.9	108–9	1718	60
7	Ac	3.75	3.66	7.76	2.55	7.94	7.98	3.00	3.15	2.1	8.7	Oil	1725	64
8[d]	H	3.75	3.67	7.62	7.24	7.61	7.85	3.39[e]	3.43[e]	2.7	8.5	110–11	1549	73
9[d]	H	3.75	3.67	7.87	7.64	7.59	8.09	3.00	3.13	2.4	8.6	107–8	1719	61
10[d,f]	H	3.61	3.66	7.98	7.18	7.34	8.01	3.03	3.03	2.4	8.4	Oil	1662	63

[a] In $CDCl_3$/TMS.
[b] All coupling constants between *para* protons were not readable; $J_{2,4} \approx J_{2,6} \approx J_{4,6}$ in the spectra of **1** and **8**–**10**
[c] *s* and *a* refer to the *syn* and *anti* position of the carbamate methyl group relative to the carbonyl oxygen.
[d] Numbering of benzene positions like in the 4-substituted compounds (**2**–**7**).
[e] Methyl group in $CSNMe_2$.
[f] 8,37 ppm (1-H, s, N–H).
[a] KBr disk.

and under reflux, just the starting material was recovered. Structures of all new compounds were proved by elemental analyses, IR and ^{1}H NMR spectra. Elemental analysis data were in agreement with the calculated ones within the acceptable error range.

Correlations between ^{1}H chemical shifts and substituents parameters were performed using scales of electric field/inductive (σ_I, σ_F and F), electronegativity (σ_x), polarizability (σ_α), steric effect (υ), resonance effects (σ_R, σ_R^0, σ_R^+, σ_R^-, and R) and polar effects (σ_m, σ_p, σ_p^0, σ_p^+, and σ_p^-) [8]. However, the SSP (Single Substituent Parameter), DSP (Dual Substituent Parameter) and DSP-NLR (Dual Substituent Parameter – Non-Linear Resonance) treatments [9, 10] did not lead to acceptable correlations.

The substituent effect on biological activity was checked by measuring the toxicity of selected examples, through lethal dose test. These guidelines are based upon the observation of Metcalf and Fukuto [11], that for a series of *N,N*-dimethylphenyl-*N′*-methylcarbamates a rough parallelism between toxicity and anti-ChE activity takes place. However, it should be noted that this is not a general rule and care should be taken to extend the particular behaviour of carbamates to other classes of compounds.

Measurements of LD_{50} were performed through intravenous injection in mice procedure [3] for three representative compounds [**1** (R=H), **3** (R=OMe) and **4** (R=Cl)]. Both electron withdrawing (Cl; $LD_{50} > 400$ mg/kg) as electron releasing substituents (MeO; LD_{50} 360 mg/kg) at the position 4, of the tested neostigmines, substantially led to a decrease in toxicity in comparison to the parent compound **1** (H; LD_{50} 90 mg/kg). These very preliminary results of biological screening of neostigmine derivatives unfortunatelly indicated that substituents at the position 4 regardless their character shall decrease the biological activity of the synthesized compounds. Therefore further screening has been abandoned.

Experimental

Melting points were determined on a MQAPF-301 apparatus without correction. IR spectra were recorded on a Perkin Elmer 1430 or FTIR 1600. ^{1}H NMR spectra were taken on a Varian Gemini-300 spectrometer at 300 MHz in $CDCl_3$/TMS. The assignment of methyl hydrogens signals of the carbamate group were made as described for compound **1** [12].

General procedure: Dimethyl sulphate (3.96 mmol) was added dropwise to a stirred solution of appropriatelly substituted carbamate **11**–**17** (or thiocarbamate, or thioncarbamate or urea derivative) (4.0 mmol) [12] in dry THF (10 ml) at room temperature. The mixture was stirred for additional 8 h and then kept at −10 °C for one day. The solid obtained was filtered and washed with diethyl ether to yield compounds **1**–**10**. Compound **4** required further stirring for another 4 h at 40 °C. Compounds **1**–**5**, **8** and **9** were obtained as white crystals and **6** as rose crystals. For the conversion of **17** to **7**, dry benzene was used as a solvent and the reaction mixture was stirred for 15 days at room temperature. Despite of several attempts to crystallize **7** with different solvents, and purification on silica gel, the compound was obtained as an yellow oil. Compound **10** required further stirring for another two days and was also obtained as a brown oil. All compounds were purified through preparative TLC [methanol (45%), chloroform (45%) and *n*-hexane (10%)].

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