International Journal of Pharmaceutics, 79 (1992) 233–242

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IJP 02646

Bispilocarpic acid monoesters as prodrugs of pilocarpine: I. Preparation and identification

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(Received 18 March 1991)
(Accepted 30 August 1991)

Key words: Prodrug; Bispilocarpic acid monoester; Synthesis; Identification; Thermospray liquid chromatography; Liquid chromatography; Mass spectrometry; NMR

Summary

Several alkyl and aralkyl bispilocarpic acid monoesters were synthesized as a new class of pilocarpine prodrugs in order tc minimize the formation of pro-moiety per mole of active pilocarpine. The compounds were prepared from pilocarpic acid by the usual esterification methods. The yield of the synthesis varied from 34 to 95%. Liquid chromatography (LC), thermospray-liquid chromatography-mass spectrometry (TSP-LC-MS), electron ionization-mass spectrometry (EI-MS) and NMR spectroscopy were applied for the identification and purity evaluation of the synthetic products.

Introduction

Many pharmaceuticals are insufficiently effective in practice because of their poor bioavailability. The prodrug approach may be a fruitful method to overcoming such difficulties. The prodrug concept involves the chemical modification of a known pharmacologically active drug into an inactive form with the aim of changing its physicochemical and pharmacokinetic properties in order to improve the biological bioavailability and drug delivery. Reversion of the prodrug to the active drug can occur either enzymatically or spontaneously.

Low bioavailability is a major problem in ocular drug delivery. Typically 1% or less of the instilled dose is ocularly absorbed (Shell, 1984; Burstein and Anderson, 1985). This is due to the corneal barrier against ocular drug absorption (Shell, 1984). The prodrug approach has been undertaken to improve corneal penetration of various ophthalmic drugs including epinephrine (Kaback et al., 1976; Mandell et al., 1978), timolol (Bundgaard et al., 1986a; Chang et al., 1987; Sasaki et al., 1988), nadolol (Duzman et al., 1982), acyclovir (Maudgal et al., 1984), terbutaline (Phipps et al., 1986), phenylephrine (Yuan and

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Bodor, 1976; Bergamini et al., 1979; Mindel et al., 1980) and pilocarpine (Bundgaard et al., 1985, 1986b; Mosher et al., 1987).

Pilocarpine is widely used in glaucoma to reduce elevated intraocular pressure. However, its ocular bioavailability is very low due to rapid solution drainage, conjunctival systemic absorption and poor corneal permeability (Sieg and Robinson, 1976; Lee and Robinson, 1979; Grass and Robinson, 1984; Urtti et al., 1985). Hence, ocular bioavailability of pilocarpine may be improved by more lipophilic prodrugs of pilocarpine.

The present paper describes the preparation and identification of bispilocarpic acid monoesters, new prodrugs of pilocarpine.

Materials and Methods

Chemicals

Pilocarpine hydrochloride was kindly provided by Huhtamäki Oy Leiras (Tampere, Finland). α,α'-Dichloro-*p*-xylene, $\alpha\alpha'$-dibromo-*m*-xylene, α,α'-dibromo-*o*-xylene, 1,3-dibromopropane, 1,4-dibromobutane, 1,5-dibromopentane, 1,6-dibromohexane, 1,7-dibromoheptane and calcium sulphate were obtained from Aldrich (Steinheim, Germany). Ethyl acetate, petroleum ether and chloroform were purchased from Merck (Darmstadt, Germany) and dimethyl sulphoxide (DMSO) was from Merck (Munich, Germany). Diethyl ether, methanol (HPLC grade) and acetonitrile (HPLC grade) were from Baker (Deventer, The Netherlands).

Synthesis of bispilocarpic acid monoester

The bispilocarpic acid monoester **1–8** (Fig. 1) were prepared by esterifying the sodium salt of pilocarpic acid (sodium pilocarpate) with alkyl or aralkyl dihalogenide. Sodium pilocarpate was synthesized as described by Järvinen et al. (1991).

1,4-Xylylene bispilocarpate (compound 6) Sodium pilocarpate (1987 mg; 8.00 mmol) was dissolved in 60 ml DMSO. α,α'-Dichloro-*p*-xylene (524 mg; 3.00 mmol) was added in DMSO over a period of 1 h. The solution was stirred at room temperature for 48–72 h and poured into 100 ml distilled water. The mixture was extracted with

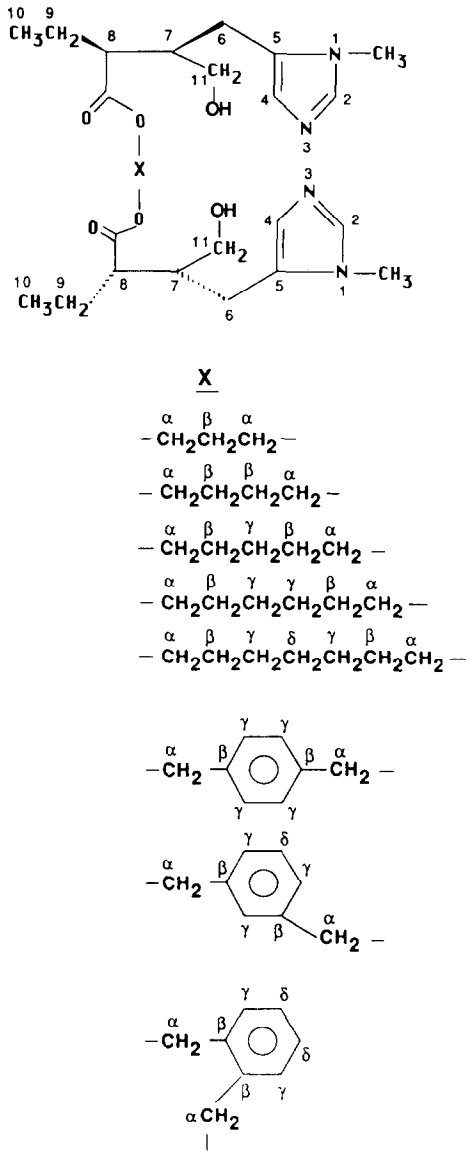

Fig. 1. Structures of bispilocarpic acid monoesters.

two 100-ml portions of chloroform. The combined chloroform extracts were washed with 100 ml of distilled water, 100 ml of 2% sodium bicarbonate solution and 100 ml of distilled water, respectively. The chloroform extracts were dried on calcium sulphate and the chloroform was removed under reduced pressure to give bispilocarpic acid 1,4-xylene monoester. The monoester was crystallized from chloroform/petroleum ether to give 770 mg (1.39 mmol) of monoester.

The yield was 42%; m.p. 170–171 °C. Formula: $C_{30}H_{42}O_6N_4$.

1,3-Xylylene bispilocarpate (compound 7) The monoester was prepared from sodium pilocarpate (1287 mg; 5.18 mmol) and α,α'-dibromo-*m*-xylene (342 mg; 1.30 mmol) according to the procedure described for compound **6**. The monoester was crystallized from ethyl acetate/ether to give 380 mg (0.69 mmol) of monoester. The yield was 53%; m.p. 116–117 °C. Formula: $C_{30}H_{42}O_6N_4$.

1,2-Xylylene bispilocarpate (compound 8) The monoester was prepared from sodium pilocarpate (1166 mg; 4.70 mmol) and α,α'-dibromo-*o*-xylene (310 mg; 1.18 mmol) as described above. The monoester was crystallized from ethyl acetate/petroleum ether to give 380 mg (0.69 mmol) of monoester. The yield was 58%; m.p. 62–64 °C. Formula: $C_{30}H_{42}O_6N_4$.

1,3-Propandiyl bispilocarpate (compound 1) The monoester was prepared from sodium pilocarpate (1021 mg; 4.11 mmol) and 1,3-dibromopropane (208 mg; 1.03 mmol) by the method described earlier. The monoester was crystallized from ethyl acetate/ether to give 330 mg (0.70 mmol) of monoester. The yield was 68%; m.p. 71–73 °C. Formula: $C_{25}H_{40}O_6N_4$.

1,4-Butandiyl bispilocarpate (compound 2) The monoester was prepared from sodium pilocarpate (1069 mg; 4.31 mmol) and 1,4-dibromobutane (233 mg; 1.08 mmol) by the procedure described earlier. The monoester was crystallized from chloroform/ether/ethyl acetate to give 185 mg (0.37 mmol) of monoester. The yield was 34%; m.p. 127–129 °C. Formula: $C_{26}H_{42}O_6N_4$.

1,5-Pentandiyl bispilocarpate (compound 3) The monoester was prepared from sodium pilocarpate (1051 mg; 4.23 mmol) and 1,5-dibromopentane (244 mg; 1.06 mmol) by the procedure described earlier. The monoester was crystallized from ethyl acetate/ether to give 395 mg (0.76 mmol) of monoester. The yield was 72%; m.p. 84–87 °C. Formula: $C_{27}H_{44}O_6N_4$.

1,6-Hexandiyl bispilocarpate (compound 4) The monoester was prepared from sodium pilocarpate (1353 mg; 5.45 mmol) and 1,6-dibromohexane (499 mg; 2.04 mmol) by the procedure described earlier. The monoester was crystallized from chloroform/petroleum ether to give 717 mg (1.34 mmol) of monoester. The yield was 66%; m.p. 115–118 °C. Formula: $C_{28}H_{46}O_6N_4$.

1,7-Heptandiyl bispilocarpate (compound 5) The monoester was prepared from the sodium pilocarpate (1994 mg; 8.03 mmol) and 1,7-dibromoheptane (518 mg; 2.01 mmol) by the procedure described earlier. The monoester was crystallized from chloroform/petroleum ether to give 1050 mg (1.91 mmol) of monoester. The yield was 95%; m.p. 117–120 °C. Formula: $C_{29}H_{48}O_6N_4$.

Melting points

The uncorrected melting points for bispilocarpic acid monoesters were determined using a Reichert thermovar (Wien, Austria) apparatus.

Liquid chromatography

Liquid chromatography (LC) was performed with a system consisting of a Beckman programmable solvent module 116, a Beckman programmable UV-detector 166 (set at 215 nm), System gold data module (Beckman, San Ramon, U.S.A.), Marathon autosampler (Spark Holland, AJ Emmen, The Netherlands) equipped with column thermostat and Rheodyne 7080-080 loop (20 μl) injector. A deactivated Supelcosil LC8-DB (15 cm × 4.6 mm i.d., 5 μm) reversed-phase column (Supelco, Bellefonte, U.S.A.) was used as a stationary phase. The mobile phase was a mixture of methanol and 0.02 M KH_2PO_4. The ratios of the solutions were optimized to each compound measured and the flow rate was 1.0 ml/min.

Thermospray liquid chromatography-mass spectrometry

Thermospray liquid chromatography-mass spectrometry (TSP-LC-MS) applications described here were carried out on VG Trio-2 quadrupole mass spectrometer (VG Masslab, Manchester, U.K.) equipped with a thermospray/plasmaspray interface probe. The instrument was operated in the thermospray ionization mode. The thermospray probe temperature was adjusted to 190 °C, the ion source temperature was 150 °C, the repeller voltage was 220 V and the other ion source conditions were optimized daily. The LC system connected to the inlet of

the thermospray interface consisted of a Beckman model 112 pump for solvent delivery and the samples were injected with a Rheodyne 7125 injector (loop volume 20 μl). The compounds were separated using a deactivated Supelcosil LC8-DB reversed-phase column (15 cm × 4.6 mm i.d., 5 μm) with an isocratic solvent system of 0.2 M ammonium acetate-acetonitrile (32–68%) and a flow rate of 1.0 ml/min.

Electron impact-mass spectrometry

The EI-mass spectra of the bispilocarpic acid monoesters were recorded on a VG 70-250SE magnetic sector mass spectrometer (VG Masslab, Manchester, U.K.). Positive EI spectra were recorded under the following conditions: electron energy, 20 or 70 eV; ionization current, 500 μA; ion source temperature, 150 °C. The resolution of the instrument was adjusted to 10000. Samples were introduced into the mass spectrometer in a glass sample holder with a direct insertion probe. The probe temperature was raised from 30 to 500 °C at a rate of 100 °C/min.

NMR spectroscopy

^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AM-250 FT/ASPECT 3000 spectrometer using a 5 mm ^{1}H/^{13}C dual probe, operating at 250 MHz for ^{1}H measurements and at 62.9 MHz for ^{13}C. For ^{1}H measurements, 20–40 mg of the compounds and for ^{13}C measurements, 40–80 mg, were added to 0.6 ml of CD_3OD with Me_4Si (0.1%) as an internal standard. The number of data points in the ^{1}H experiment was 32K, total relaxation time 16 s, number of scans 64 and pulse angle 45°. Decoupled ^{13}C-NMR spectra were recorded using a composite pulse sequence with 64K data points, 10 s relaxation time and 90° pulse angle. The COSY spectra (^{1}H-^{1}H correlated) were acquired as 256 · 512 matrices with zero filling to 512 · 512.

Results and Discussion

Synthesis of bispilocarpic acid monoesters

Bundgaard et al. (1986c) reported that various esters of pilocarpic acid are capable of undergoing a spontaneous lactonization in neutral or alkaline aqueous solution. The methods used in the opening of the lactone ring generally involve basic hydrolysis in aqueous solution. In addition to the formation of pilocarpic acid, pilocarpine epimerizes to isopilocarpine, which further hydrolyzes to isopilocarpic acid (Chung et al., 1970; Nunes and Brochmann-Hanssen, 1974). Epimerization of pilocarpine increases rapidly at higher temperatures (Bundgaard and Hanssen, 1982). We found that the amount of isopilocarpic acid formed during the synthesis of pilocarpic acid varied from 10 to 18%. In order to minimize the epimerization of pilocarpine, synthesis of the sodium salt of pilocarpic acid was performed at 0–4 °C.

The yields for the syntheses of bispilocarpic acid monoesters **1–8** varied from 34 to 95%. The low yield of some compounds may be due to the formation of a quaternary derivative during the alkylation of pilocarpic acid salt. However, quaternary by-products remain in the aqueous DMSO phase during the isolation procedure. The major impurity with the synthesized compounds was the corresponding monopilocarpic acid monoester, especially when an alkyl chloride was used as a reagent. We found that Br^- is a better leaving group than Cl^-, particularly in the synthesis of compounds with an aliphatic spacer where the alkyl bromide must be used as a reagent in order to obtain higher yields of the compounds.

EI-mass spectrometry

To obtain structural information on the synthesized compounds, analysis was performed by mass spectrometry using electron impact ionization (EI). The representative EI (70 eV) mass spectrum of bispilocarpic acid monoester is shown in Fig. 2. Two major fragments were observed in the mass spectrum and furthermore no molecular ion was evident. Fragment ion m/z 208 may be formed by loss of spacer compound following the cyclization of the fragment to pilocarpine. The base peak at m/z 95 corresponds to the imidazole ring, a fragment from bispilocarpic acid monoester. The mass spectra of all the bispilocarpic acid monoesters studied demonstrated two major fragment ions: that at m/z 95 as a base peak and

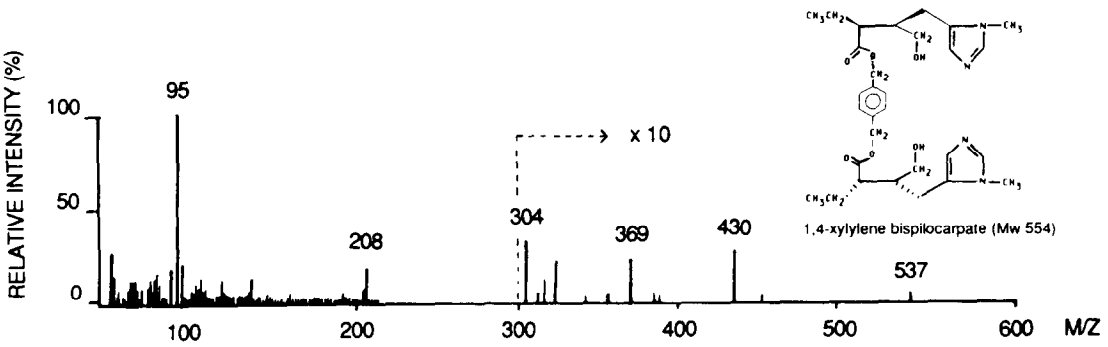

Fig. 2. Electron impact mass spectrum (70 eV) of 1,4-xylylene bispilocarpate.

the ion of pilocarpine at m/z 208. Recording of the mass spectra of the bispilocarpic acid monoesters required the use of high probe temperature. Presumably, according to the thermal decomposition of the compounds, no molecular ion was observed even at an energy of ionization of 18 eV. Hence, other ionization techniques are necessary in order to verify the molecular weight.

Identification by LC-UV and TSP-LC-MS

The reversed phase LC-UV procedure was developed to provide a suitable method for evaluation of the purity of the synthetic product and for determination of the physicochemical properties of the monoesters. TSP-LC-MS was employed in the characterization of the new bispilocarpic acid monoesters, including verification of the molecular weights.

The synthetic products were separated on a reversed-phase Supelcosil LC8-DB column eluted with methanol-phosphate buffer. This method resolves pilocarpine, pilocarpic acid, and monopilocarpic acid monoester from bispilocarpic acid monoester, and was used to monitor the purity of

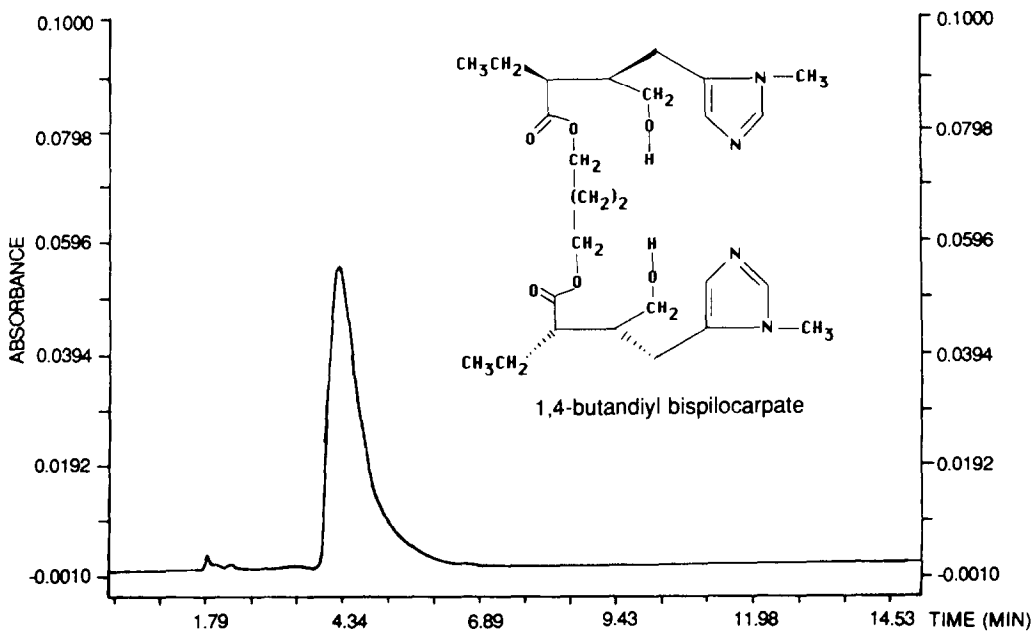

Fig. 3. LC-UV chromatogram of the synthetic product of 1,4-butandiyl bispilocarpate. LC conditions are described in the text.

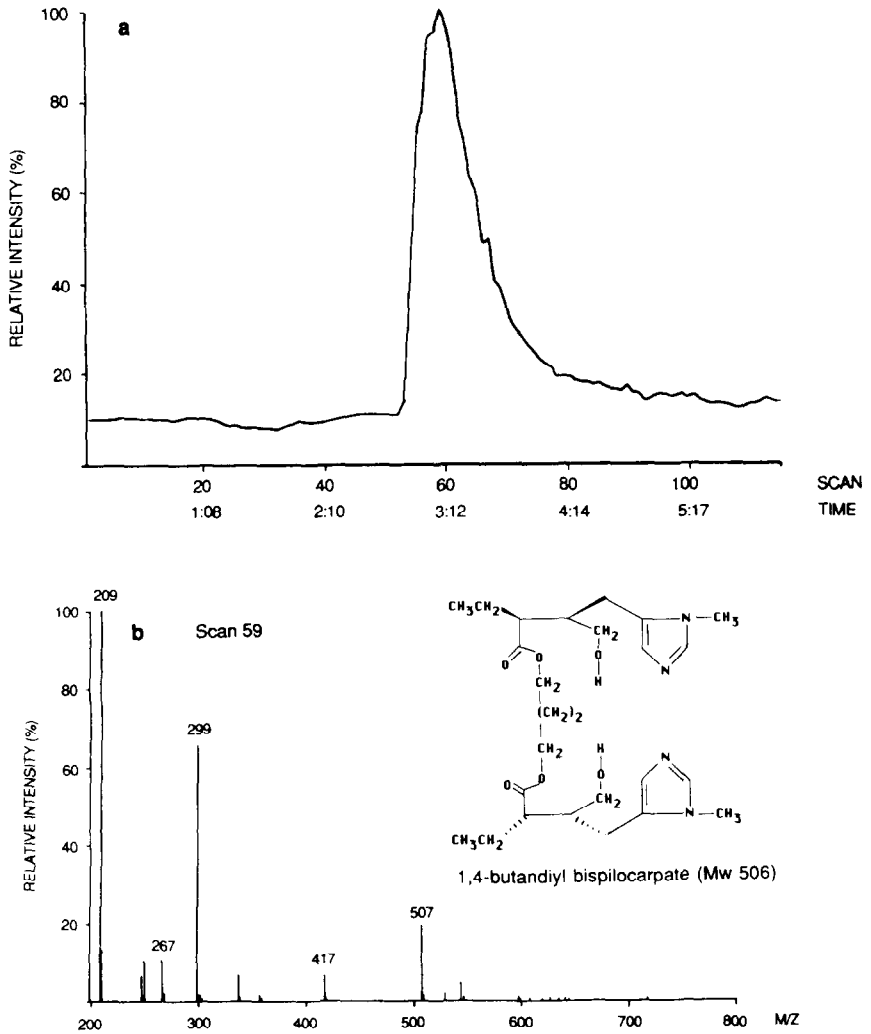

Fig. 4. TSP-LC-MS total ion profile of 1,4-butandiyl bispilocarpate (a) and positive ion TSP mass spectrum from scan 59 (b).

TABLE 1

Relative abundance of the ions in the TSP spectra of bispilocarpic acid monoesters

Ester	$[M + H]^+$	$[B + H]^+$	$[C + H]^+$	Other fragment ions
1	493 (25%)	285 (73%)	209 (100%)	417 (7%), 323 (9%), 267 (9%), 250 (10%)
2	507 (18%)	299 (66%)	209 (100%)	417 (6%), 337 (6%), 267 (11%), 250 (11%)
3	521 (15%)	313 (73%)	209 (100%)	417 (6%), 351 (9%), 267 (11%), 250 (12%)
4	535 (18%)	327 (77%)	209 (100%)	417 (8%), 365 (16%), 266 (23%), 250 (16%)
5	549 (19%)	341 (67%)	209 (100%)	417 (7%), 379 (12%), 267 (12%), 250 (17%)
6	555 (12%)	347 (46%)	209 (100%)	417 (3%), 384 (4%), 267 (10%), 250 (5%)
7	555 (7%)	347 (46%)	209 (100%)	417 (7%), 385 (8%), 267 (10%), 250 (10%)
8	555 (11%)	347 (47%)	209 (100%)	417 (7%), 385 (8%), 267 (9%), 250 (14%)

B and C correspond to the structures in Fig. 5.

the compounds. Fig. 3 shows a typical LC chromatogram obtained from a synthetic product of 1,4-butandiyl bispilocarpate (compound **2**).

Satisfactory chromatographic separation of the bispilocarpic acid monoesters from the supposed impurities (the corresponding monopilocarpic acid monoester and pilocarpine) in the TSP-LC system was achieved on isocratic elution with 0.2 M ammonium acetate and acetonitrile (32–68%) as mobile phase at a flow rate of 1.0 ml/min. In TSP ionization, analyte ions are produced upon nebulization of an aqueous solution containing ammonium acetate (Arpino, 1990). A deactivated Supelcosil LC8-DB column resulted in a satisfactory peak shape for bispilocarpic acid monoester although the surface of the thermospray capillary absorbs the compounds and slight peak broadening was observed. Fig. 4a shows the total ion current LC-MS profile from 1,4-butandiyl bispilocarpate (compound **2**). The TSP mass spectrum of the main peak (scan 59) is also shown in Fig. 4b. The protonated molecular ion $[M + 1]^+$ was observed at m/z 507. Two important fragment ions were m/z 299 and the base peak at m/z 209, corresponding presumably to structures B and C (Fig. 5), respectively. Similar decomposition of bispilocarpic acid monoester was also found to occur in aqueous solution (Järvinen et al., 1992).

The TSP mass spectra of all the other bispilocarpic acid monoesters studied displayed similar fragment ions. The relative abundance of selected fragments ions is listed in Table 1.

Mild ionization of TSP is ideally suited to the identification of bispilocarpic acid monoesters as some structural information is obtained in addition to the value of the molecular weight. The TSP method would be optimal if the mobile phase used in the LC-UV method were compatible with the TSP-LC-MS procedure. In the described application, non-volatile phosphate buffer (LC-UV) was replaced by volatile ammonium acetate buffer (used in LC-MS) without any marked influence on the separation.

NMR spectroscopy

Pertinent NMR data are required together with other details for the identification of drug

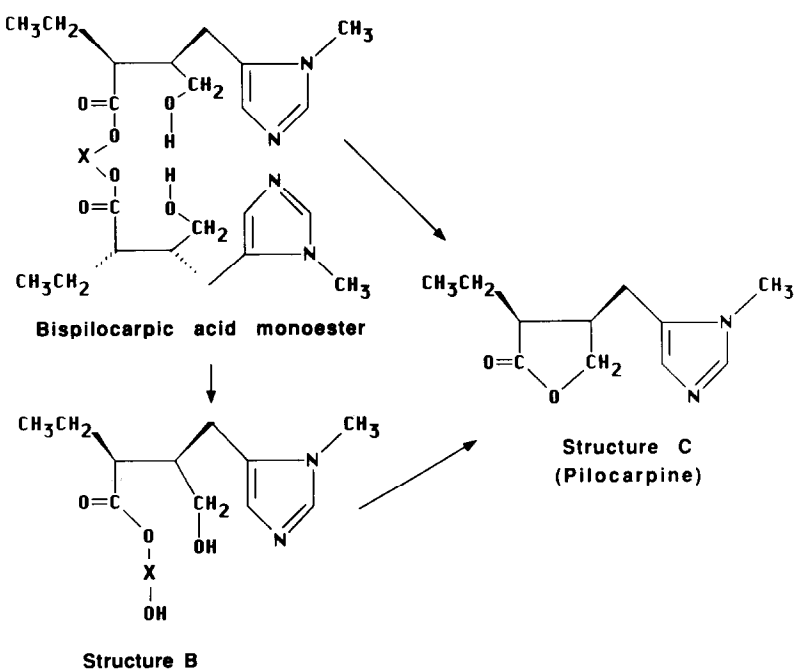

Fig. 5. Decomposition of bispilocarpic acid monoester in aqueous solution and in TSP analysis.

TABLE 2

Proton chemical shifts for compounds 1–8 (ppm)

Proton	Compound							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
N-Me	3.60	3.60	3.60	3.60	3.59	3.48	3.48	3.48
2	7.50	7.50	7.50	7.49	7.50	7.47	7.47	7.47
4	6.72	6.73	6.73	6.73	6.73	6.69	6.70	6.70
6_α	2.72	2.73	2.73	2.72	2.72	2.65	2.66	2.66
6_β	2.53	2.52	2.52	2.51	2.50	2.42	2.43	2.42
7	1.99	2.00	1.99	1.99	1.99	1.97	1.97	1.97
8	2.49	2.47	2.48	2.47	2.47	2.53	2.52	2.53
9	1.68	1.68	1.68	1.67	1.68	1.67	1.67	1.67
10	0.89	0.89	0.89	0.88	0.88	0.84	0.85	0.85
11	3.54	3.54	3.55	3.53	3.53	3.51	3.52	3.52
R_α	4.19	4.08	4.07	4.05	4.05	5.09	5.09	5.24
R_β	2.08	1.81	1.64	1.65	1.64	–	–	–
R_γ	–	–	1.52	1.41	1.64	7.39	[a]	7.34
R_δ	–	–	–	–	1.37	–	[a]	7.34

[a] 7.44 ppm broad singlet (^{1}H) and 7.35 ppm broad singlet (^{3}H).

substances. In Table 2, we report all the important ^{1}H chemical shifts for compounds **1–8** and Table 3 lists the ^{13}C chemical shifts for compounds **1**, **2**, **4** and **5**. The measured ^{1}H data are consistent with those reported for pilocarpic acid diesters by Järvinen et al. (1991). Two-dimensional COSY NMR spectroscopy enabled us to correlate fully the proton chemical shifts. Both ^{1}H and ^{13}C spectra clearly indicated good agreement with chemical shifts and structures.

The limit of detection of impurities and solvent residues was lower than 0.5 mol%. The impurity analysis was based on the NMR signals from the methyl group in the ethyl (CH_3CH_2-) side chain and signals in the aromatic region. Since both of these groups are sensitive to stereochemical effects in their surroundings other pilocarpine derivatives can be quantitated satisfactorily. Some of the compounds examined (e.g. **8**) decomposed fairly rapidly in solution and, consequently, the NMR data do not allow accurate estimation of the degree of the total impurities.

TABLE 3

Carbon chemical shifts for compounds 1, 2, 4 and 5 (ppm)

Carbon	Compound			
	1	**2**	**4**	**5**
N-Me	31.70	31.72	31.71	31.66
2	138.97	138.95	138.96	138.95
4	127.44	127.44	127.45	127.40
5	131.69	131.71	131.72	131.67
6	24.35	24.41	24.40	24.40
7	43.26	43.26	43.28	43.30
8	49.31	49.33	49.41	49.43
C=O	176.96	177.07	177.17	177.21
9	23.51	23.47	23.50	23.51
10	12.35	12.37	12.38	12.38
11	61.25	61.26	61.20	61.19
R_α	62.25	64.81	64.40	65.49
R_β	32.59	30.48	29.63	29.66
R_γ	–	–	26.74	27.03
R_δ	–	–	–	26.83

Conclusions

The coupling of two pilocarpic acid molecules via a spacer chain is an efficient and feasible method of preparing a new type prodrug of pilocarpine, in which the amount of the pro-moiety is minimized. This is important from the viewpoint of safety. Various synthetic methods were evaluated with respect to the preparation of new prodrugs in reasonable yields. Thermospray liquid

chromatography was found to be a very useful technique in the characterization of the compounds along with NMR spectroscopy, especially when EI-mass spectrometry could not be used in the determination of the elemental composition. The unambiguous identification and purity evaluation of the bispilocarpic acid monoesters could be accomplished only when TSP-LC-MS, LC-UV and ^{1}H- and ^{13}C-NMR spectroscopy were used.

Acknowledgements

The authors would like to thank Huhtamäki OY Leiras (Finland), the Technology Development Centre (Finland), Jenny and Antti Wihuri Foundation and the Finnish Cultural Foundation for financial support. We also thank Leiras for research collaboration. We are grateful to Mr Jukka Knuutinen and Ms Päivi Varis for skillful technical assistance.

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