Chemoenzymatic Synthesis of the Eight Stereoisomeric Muscarines

Marco De Amici,[1a] Carlo De Micheli,*[1a] Giorgio Molteni,[1a] Davide Pitrè,[1a] Giacomo Carrea,*[1b] Sergio Riva,[1b] Sandro Spezia,[1b] and Lucia Zetta[1c]

Istituto di Chimica Farmaceutica dell'Università, Viale Abruzzi 42, I-20131 Milano, Italy, Istituto di Chimica degli Ormoni del CNR, Via M.Bianco 9, I-20131 Milano, Italy, and Istituto di Chimica delle Macromolecole del CNR, Via Ampère 56, I-20131 Milano, Italy

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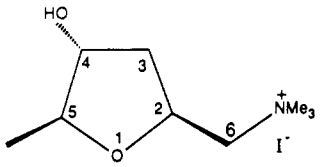
Efficient syntheses of the eight stereoisomers of muscarine have been accomplished by dehydrogenase-catalyzed reduction of iodo ketones (±)-**3a** and (±)-**3b**. 3α,20β-Hydroxysteroid dehydrogenase from *Streptomyces hydrogenans* exhibited high enantiomeric and diastereotopic selectivity for (±)-**3a**, yielding an equimolar mixture of iodo alcohol (−)-**4** (2*S*,4*S*,5*S*) (96% ee) and iodo ketone (+)-**3a** (2*R*,5*R*) (96% ee) which was reduced by sodium borohydride to a mixture of (+)-**4** and (+)-**5**. 3β,17β-Hydroxysteroid dehydrogenase from *Pseudomonas testosteroni* reduced (±)-**3b** with high diastereotopic selectivity to give an equimolar mixture of iodo alcohols (+)-**6** (2*R*,4*S*,5*S*) (>99% ee) and (−)-**7** (2*S*,4*S*,5*R*) (81% ee). Synthesis of the remaining iodo alcohols [(−)-**5**, (−)-**6**, and (+)-**7**] was achieved by applying the Mitsunobu procedure to (−)-**4**, (−)-**7**, and (+)-**6**. The enantiomeric excess of intermediates **4-7** was determined by HPLC analysis of the (*R*)-(+)-MTPA esters. The chiral iodo alcohols **4-7** were then transformed into the final derivatives by conventional chemical manipulations.

Introduction

Muscarine (**1**: OH^- instead of I^-) is one of the most widely studied alkaloids due to its presence in a variety of poisonous mushrooms, i.e., *Amanita phalloides* (death cap), *Amanita muscaria* (fly agaric), and certain species of *Inocybe* and *Clitocybe*.[2] It was isolated from the fly agaric in 1954.[3] Its structure was established by X-ray

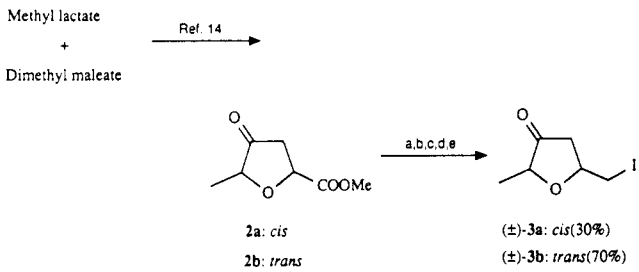


(+)-**1** (2S,4R,5S)

analysis,[4] and its pharmacological properties have been thoroughly investigated.[5] Muscarine is a selective agonist of acetylcholine on smooth muscles of the gastrointestinal tract, eye, exocrine glands, and heart. As a consequence, the receptor responses to the physiological neurotransmitter acetylcholine in those tissues were termed muscarinic effects. Recently, pharmacological investigations with selective antagonists evidenced distinct subtypes of muscarinic receptors.[6] The receptors in central nervous system and peripheral ganglia (M_1) are distinguishable from the receptors on the cardiac cells (M_2) and also different from those on smooth muscles and exocrine glands (M_3). There is renewed interest in the muscarinic field because of the discovery of a relationship between cholinergic deficits in cortical and hippocampal areas and the pathology of Alzheimer's disease.[7]

As an extension of our study on the structure–activity relationship of muscarinic ligands,[8,9] we became interested in the pharmacological selectivity of the muscarinic diastereomers toward all three muscarinic receptor subtypes. For such a purpose, we needed a synthetic strategy suited for producing a large number of muscarine stereoisomers in high enantiomeric excess. The synthetic routes reported so far were designed to prepare one or a few isomers of muscarine.[10] This paper reports a preparative route to the enantiomers of muscarine **1** and its stereoisomers **8**, **9**, **10** via an asymmetric reduction of iodo ketones (±)-**3a** and (±)-**3b**, catalyzed by 3α,20β-hydroxysteroid dehydrogenase from *Streptomyces hydrogenans* (20β-HSDH) and 3β,17β-hydroxysteroid dehydrogenase from *Pseudomonas testosteroni* (β-HSDH). The intermediates were then transformed into the final derivatives by conventional chemical manipulations. This research further extends and emphasizes the well-known usefulness and versatility of dehydrogenases in organic synthesis.[11] Moreover, it shows that hydroxysteroid dehydrogenases, which have been used in regio- and stereospecific redox transformations of keto–hydroxy groups of bile acids[12] and

Scheme I[a]



[a] (a) $MeOH/(MeO)_3CH/H_2SO_4$; (b) $LiAlH_4/Et_2O$; (c) $MeSO_2Cl/NEt_3$; (d) CF_3COOH/H_2O; (e) NaI/acetone.

(1) (a) Istituto di Chimica Farmaceutica, Milano. (b) Istituto di Chimica degli Ormoni, Milano. (c) Istituto di Chimica delle Macromolecole, Milano.
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Table I. ^1H and ^{13}C NMR Data of Compounds 1, 3–13[a,b]

compd	H-5	H-4	H-3	H-3′	H-2	H-6	H-6′	5-Me	$J_{4,5}$	$J_{3,4}$	$J_{3',4}$	$J_{3,3'}$	$J_{2,3}$	$J_{2,3'}$	$J_{2,6}$	$J_{2,6'}$	$J_{6,6'}$
3a	3.99		2.67	2.31	4.14	3.41		1.34				18.1	6.1	9.9	5.3[d]		
3b[c]	4.21		2.70	2.46	4.44	3.38	3.35	1.27				18.1	6.7	6.9	4.6	7.0	10.3
4	3.98	3.90	2.44	1.81	4.21	3.45	3.36	1.33	3.6	5.9	1.4	14.4	8.5	5.4	6.4	4.5	10.0
5	3.92	3.99	2.03	1.88	4.07	3.28	3.22	1.23	3.3	3.0	6.2	13.3	6.2	8.7	4.6	6.3	10.1
6	4.22	4.14	2.23	1.91	4.28	3.29	3.25	1.25	2.8	0.5	4.6	13.5	6.3	8.7	4.0	6.9	10.0
7	4.09	4.05	2.45	1.83	4.21	3.34		1.20	4.1	6.3	5.3	13.5	7.7	5.3	6.3[d]		
1[e]	3.96	4.03	2.01	1.91	4.57	3.49	3.39	1.11	2.5	2.3	5.7	13.7	6.3	9.6	1.8	9.2	14.0
8[e]	3.85	4.14	2.51	1.51	4.37	3.46	3.42	1.13	3.5	6.0	1.9	14.3	8.7	5.7	8.2	3.3	13.9
9[e]	4.01	4.14	2.14	1.85	4.65	3.43	3.30	1.13	2.6	0.5	4.9	14.1	7.2	8.7	9.6	1.8	14.0
10[e]	3.97	3.97	2.51	1.56	4.63	3.58	3.34	1.11	4.1	6.0	5.4	13.7	8.0	5.8	9.8	1.3	14.0
11	4.28	5.14	2.28	2.05	4.17	3.34	3.28	1.36	2.5	1.6	6.3	13.8	5.4	9.8	4.7	6.3	10.1
12	4.42	5.18	2.65	2.15	4.40	3.38	3.34	1.28	2.8	6.8	3.2	14.3	7.2	4.5	5.5	8.0	9.8
13	4.42	5.57	2.42	2.13	4.35	3.35	3.32	1.30	3.4	1.1	5.0	14.1	6.2	9.0	4.2	7.0	10.0

compd	C-2	C-3	C-4	C-5	C-6	C-Me	C-NMe_3
1[e]	74.85	40.53	78.13	86.91	73.51	22.11	57.09
8[e]	73.95	41.96	74.16	83.43	73.00	16.15	56.82
9[e]	73.93	41.82	74.78	81.84	72.23	16.03	56.96
10[e]	74.16	40.46	78.15	84.63	72.58	20.41	56.92

[a] For the sake of clarity, derivatives 1, 3–13 have been numbered as shown for muscarine 1. [b] Chemical shifts in δ and coupling constants in hertz; in the case of geminal protons, that resonating at higher field is designated with a prime. The carbon signals have been assigned on the basis of ^{13}C–^1H heterocorrelated spectra. [c] $J_{5,3'} = 1.0$ Hz. [d] $(J_{2,6} + J_{2,6'})/2$. [e] Solvent: D_2O.

Table II. Enzymatic Reduction of (±)-3a and (±)-3b[a]

enzyme	substrate	time (h)	% tranf	substrate	time (h)	% transf
20β-HSDH	(±)-**3a**	24	52	(±)-**3b**	24	ND[b]
β-HSDH	(±)-**3a**	24	20	(±)-**3b**	24	>95
3α-HSDH	(±)-**3a**	24	30	(±)-**3b**	24	ND
7α-HSDH	(±)-**3a**	24	ND[b]	(±)-**3b**	24	ND
12α-HSDH	(±)-**3a**	24	ND	(±)-**3b**	24	ND
TBADH	(±)-**3a**	24	ND	(±)-**3b**	24	ND
HLADH	(±)-**3a**	24	ND	(±)-**3b**	24	ND

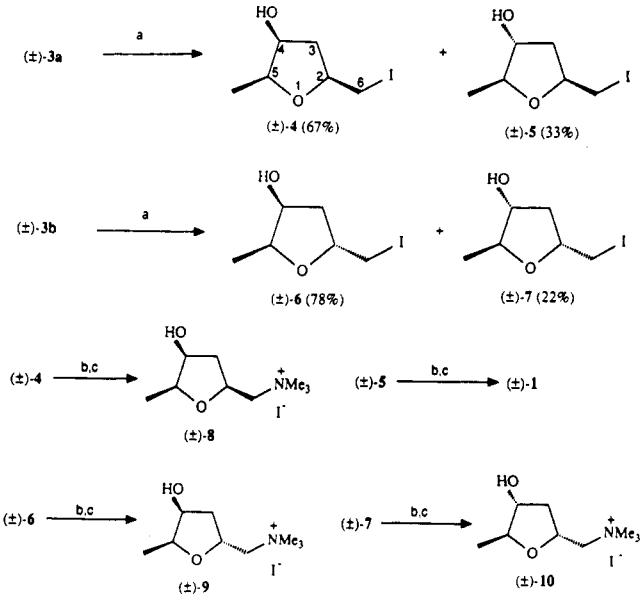
[a] The substrates (40 mM) were incubated with the various dehydrogenases (2.5 units/mL), at pH 6.6, 25 °C. NADH or NADPH (with the NADP-dependent 12α-HSDH and TBADH) were regenerated at the expense of formate or glucose, respectively.[12a] [b] ND: not detectable.

neutral steroids,[13] work equally well on "unnatural" substrates such as **3a** and **3b**.

Results and Discussion

A 30:70 mixture of stereoisomeric iodo ketones (±)-**3a** and (±)-**3b** was prepared from (±)-**2a** and (±)-**2b** by standard transformations of their ester functionality (Scheme I). In turn, intermediates (±)-**2a** and (±)-**2b** were prepared by addition of methyl lactate to dimethyl maleate or fumarate, according to the procedure previously reported by Hardegger et al.[14] (±)-**3a** and (±)**3b** can be separated into pure components by flash chromatography on a silica gel column. The structures of (±)-**3a** and (±)-**3b** were assigned by ^1H NMR spectroscopy (Table I). As previously observed for related structures,[8] the following characteristic spectral features were diagnostic in the stereochemical assignment. (a) H-5 and H-2 of the trans isomer **3b** absorb at lower field (δ 4.21 and 4.44) than those of the cis isomer **3a** (δ 3.99 and 4.14). (b) The 5-Me signal of the trans isomer **3b** appears at higher field (δ 1.27) than that of the cis isomer (δ 1.34). (c) The spectrum of the trans isomer **3b** solely shows a long-range coupling constant (1.0 Hz) between H-5 and H-3′. The assignment of structure to (±)-**3a** and (±)-**3b** was unequivocally established by comparing the physical properties of the couple muscarine (**1**)–epimuscarine (**8**) and the couple epiallomuscarine (**9**)–allomuscarine (**10**) with those previously reported[15] (see the Experimental Section). It is worth

Scheme II[a]



[a] (a) $NaBH_4$/EtOH; (b) $NHMe_2$/MeOH; (c) MeI/Et_2O.

pointing out that the cis–trans relationship between the 4-hydroxy and the 5-methyl groups of derivatives **1**, **8**, **9**, **10** can also be deduced from the relative values of the chemical shifts of C-4 and C-Me. Owing to steric effects the C-Me and C-4 carbon atoms in the cis isomers **8** and **9** are shielded by ca. 5 ppm relative to the trans isomers **1** and **10**.

To test the feasibility of our approach to the synthesis of the muscarine stereoisomers, we first reduced (±)-**3a** and

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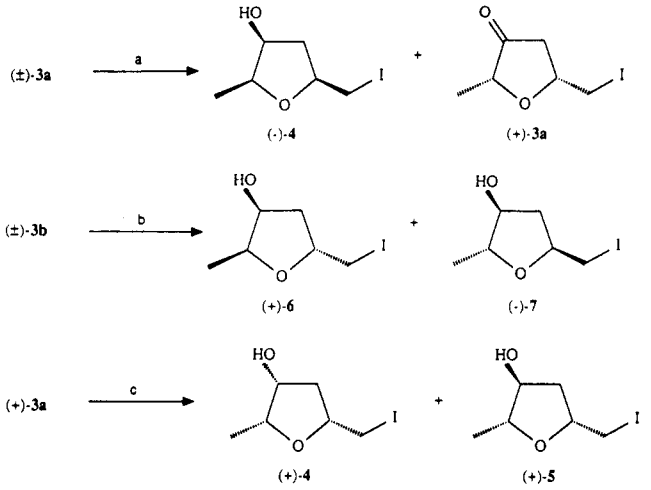
Scheme III[a]



[a] (a) 20β-HSDH, K_2HPO_4 0.05 M, pH 7; (b) β-HSDH, K_2HPO_4 0.05 M, pH 6.7; (c) $NaBH_4$/EtOH.

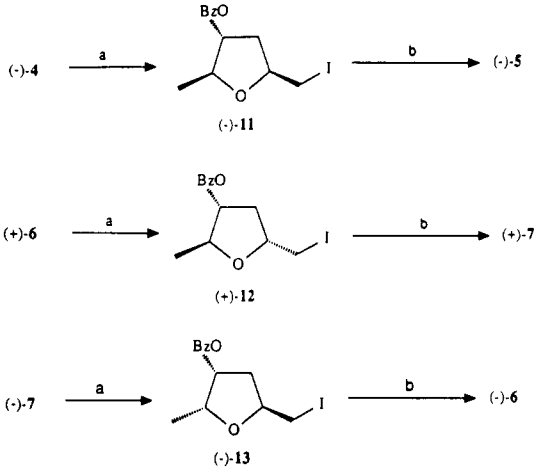
(±)-**3b** with sodium borohydride separately. In this way we prepared the couples of iodo alcohols **4**–**5** (67:33) and **6**–**7** (78:22), which were separated into pure components by flash chromatography and then treated sequentially with dimethylamine and methyl iodide to provide ammonium salts (±)-**8**, (±)-**1**, (±)-**9**, and (±)-**10** (Scheme II)

To tackle the synthesis of optically active iodo alcohols **4**–**7**, we submitted racemic iodo ketones **3a** and **3b** to different enzyme-catalyzed reductions. Table II reports the results obtained with a series of hydroxysteroid dehydrogenases (HSDH) and with the alcohol dehydrogenases from *Thermoanaerobium brockii* (TBADH) and horse liver (HLADH). The data of Table II clearly evidence that hydroxysteroid dehydrogenase from *Streptomyces hydrogenans* (20β-HSDH) is the most efficient catalyst for the transformation of (±)-**3a** and that hydroxysteroid dehydrogenase from *Pseudomonas testosteroni* (β-HSDH) is the most appropriate enzyme for the reduction of (±)-**3b**.[16] The 20β-HSDH-catalyzed reduction[17] of (±)-**3a** showed remarkable enantio- and diastereotopic selectivity, yielding an equimolar mixture of iodo alcohol (-)-**4** and iodo ketone (+)-**3a** (Scheme III). Preparation of (-)-**4** and (+)-**3a**, both with high enantiomeric excess (96%), was accomplished by a two-step sequence. First the reduction process was stopped at 44% conversion, and the mixture was column chromatographed to separate iodo alcohol (-)-**4** from the remaining iodo ketone, which was resubmitted to enzymatic reduction until a GLC analysis showed 55% global conversion. Enantiomer (+)-**3a**, collected in a second column chromatography, was then reduced with sodium borohydride and yielded a 67:33 mixture of diastereomeric iodo alcohols (+)-**4** and (+)-**5** (Scheme III). These intermediates were separated by column chromatography and fully characterized by ^1H NMR, specific rotation (see the Experimental Section), and their transformation into the corresponding muscarine stereoisomers. The enantiomeric excesses of (-)-**4**, (+)-**4**, and (+)-**5**, were ascertained by HPLC analysis

(16) The K_m value for (±)-**3a** of 20β-HSDH was 7.5 mM and the V_{max} value was about 20% of that found with cortisone (17α,21-dihydroxy-4-pregnene-3,11,20-trione), the reference substrate. The K_m value for (±)-**3b** of β-HSDH was 4.9 mM and the V_{max} value was about 90% of that found with epiandrosterone (3β-hydroxy-5α-androstan-17-one).

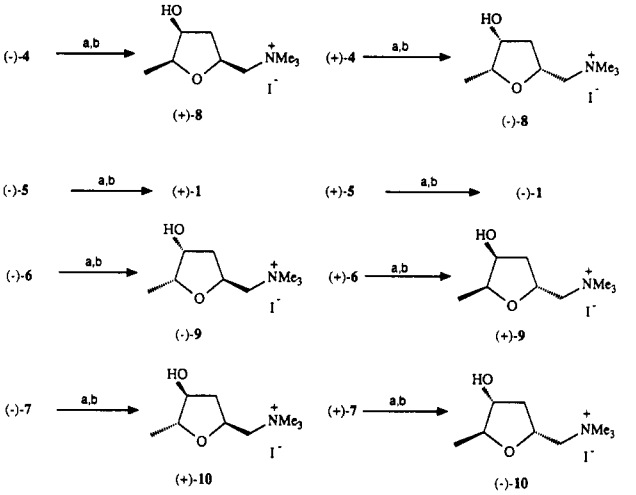
(17) NADH, the hydrogen donor for the 20β-HSDH- and β-HSDH-catalyzed reductions of (±)-**3a** and (±)-**3b** was continuously in situ regenerated from NAD at the expense of formate in a reaction catalyzed by formate dehydrogenase.[12a]

Scheme IV[a]



[a] (a) Ph_3P/DEAD/benzoic acid; (b) K_2CO_3/water–methanol.

Scheme V[a]



[a] (a) $NHMe_2$/MeOH; (b) MeI/Et_2O.

of the corresponding (+)-MTPA esters (see the Experimental Section).

The β-HSDH-catalyzed reduction[17] of (±)-**3b** (Scheme III) was not enantiomer-selective since the two enantiomers were reduced at a comparable rate. Nevertheless, the reduction process shows high facial selectivity; the enzyme directs hydride transfer to the *re* face of the carbonyl moiety of both the enantiomers, leading to the formation of the stereoisomeric iodoalcohols (+)-**6** and (-)-**7** in high ee (99 and 81%, respectively) and with the same *S* configuration at the new chiral center. The different ee values of (+)-**6** and (-)-**7** derive from a different degree of selectivity of the enzyme for the two enantiomers of **3b**. The reduction of the 2*S*,5*R* enantiomer is completely diastereoselective and produces only (-)-**7**, whereas the reduction of the 2*R*,5*S* enantiomer is highly diastereoselective, yielding (+)-**6** contaminated with a minor amount of (+)-**7**.

The synthesis of the remaining three iodo alcohols [(-)-**5**, (+)-**7**, and (-)-**6**] required inversion of configuration at C-4 of substrates (-)-**4**, (+)-**6**, and (-)-**7**, respectively. Treatment of intermediate (-)-**4** with triphenylphosphine (TPP), diethyl azodicarboxylate (DEAD), and benzoic acid at 0 °C, according to the Mitsunobu protocol,[10d,18] cleanly produced benzoate (-)-**11** with complete inversion of con-

(18) Mitsunobu, O. *Synthesis* **1981**, 1–28.

figuration at C-4, 86% yield (Scheme IV). Benzoate (−)-**11** was then efficiently hydrolyzed to (−)-**5** by treatment with potassium carbonate in water–methanol (1:1). The same methodology applied to (+)-**6** and (−)-**7** yielded (+)-**12** and (−)-**13** which, in turn, were transformed into (+)-**7** and (−)-**6** (Scheme IV). Finally the eight stereomeric iodo alcohols **4**–**7** were reacted with dimethylamine and methyl iodide to produce all the possible muscarine stereoisomers (Scheme V).

In conclusion, this work further evidences the successful application of enzyme-catalyzed transformations to the synthesis of chiral synthons in high enantiomeric excess. This report shows especially that enzymes such as 20β-HSDH and β-HSDH which, up to now, have been used almost exclusively[19] for redox processes of "natural" substrates such as steroids[12,13] work with high efficiency on "unnatural" substrates such as (±)-**3a** and (±)-**3b**.

Experimental Section

Materials and Methods. 3α,20β-Hydroxysteroid dehydrogenase (20β-HSDH), 3β,17β-hydroxysteroid dehydrogenase (β-HSDH), 3α-hydroxysteroid dehydrogenase (3α-HSDH), 7α-hydroxysteroid dehydrogenase (7α-HSDH), alcohol dehydrogenase from *Thermoanaerobium brockii* (TBADH) (purified powder), horse liver alcohol dehydrogenase (HLADH), NAD, and NADP were purchased from Sigma. Formate dehydrogenase was bought from Boehringer and 12α-hydroxysteroid dehydrogenase (12α-HSDH) was extracted from *Clostridium* group P.[20] Organic solvents were reagent grade. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ or D_2O solution at 270 and 50.32 MHz, respectively. The heterocorrelated ^{13}C–^1H spectra were obtained by using a data matrix of 1024(F2) × 236(F1) with a spectral width of 5100 Hz in the carbon dimension and 400 Hz in the proton dimension. GLC analyses were carried out on a 5-m HP1 capillary silica gel column coated with methylsilicone gum. N_2 was used as the carrier gas at 30 mL/min. HPLC analyses were performed on a chromatograph equipped with a UV detector (λ = 254 nm) and a Whatman Partisil 10 column (250 mm length, 4.6 mm i.d.); a mixture *n*-hexane/ethyl acetate (9:1) was used as the eluent at the flow rate of 1.0 mL/min. Retention times (t_R) are expressed in minutes. Rotatory power determinations were carried out with a polarimeter, coupled with a thermostat. Melting points and molar purities were obtained from the DSC curves, recorded with a differential scanning calorimeter under the following conditions: sample weight about 2 mg; heating rate 2 °C/min. Indium was used as the reference compound. Liquids were characterized by the oven temperature for Kugelrohr distillations. (*R*)-(+)-MTPA esters were prepared according to the procedure described previously.[21]

***cis*- and *trans*-5-Methyl-2-(iodomethyl)-4(2*H*)-dihydrofuranones [(±)-3a and (±)-3b]. A.** In a 500-mL Erlenmeyer flask, equipped with a magnetic stirrer and a refluxing condenser, a mixture of keto esters (±)-**2a** and (±)-**2b**[14] (27 g, 0.17 mol), trimethyl orthoformate (27 g, 0.25 mol), concentrated sulfuric acid (8 μL), and methanol (200 mL) were refluxed overnight. The solution was treated with ether (150 mL), washed with a saturated $NaHCO_3$ solution (3 × 30 mL), and dried over anhydrous sodium sulfate. After evaporation of the solvent, the residue was distilled at 110 °C (0.5 mmHg) to yield 31 g (89%) of the corresponding dimethyl ketals, which were directly submitted to the following reaction.

B. In a flask equipped with a magnetic stirrer, a dropping funnel, and a refluxing condenser, lithium aluminum hydride (5 g) and anhydrous THF (250 mL) were charged. A solution of the dimethyl ketals (31 g, 0.15 mol) in anhydrous THF (200 mL) was added dropwise at room temperature. The reaction mixture was stirred at 25 °C until disappearance of the starting material (about 2 h). Water was cautiously added, and the resulting slurry was filtered through Celite and washed with ether. The organic phase was dried and evaporated, and the residue was Kugelrohr distilled at 110 °C (1 mmHg). Yield: 24 g (90%). The mixture of alcohols was directly used in the following step.

C. A 500-mL Erlenmeyer flask, equipped with a magnetic stirrer and a septum, was charged with the above-prepared mixture of alcohols (24 g, 0.14 mol) and dichloromethane (150 mL). To the solution cooled at 0 °C, triethylamine (70 mL, 0.50 mol) and methanesulfonyl chloride (24 mL, 0.31 mol) were added sequentially. The suspension was stirred at 0 °C until disappearance of the starting material and then poured into water. The organic layer was washed with 2 N HCl and aqueous $NaHCO_3$ and dried over anhydrous sodium sulfate. Evaporation of the solvent under vacuum gave a mixture of crude mesylates; yield 30 g (87%). No contaminants were detected by TLC (cyclohexane/ethyl acetate, 3:2).

D. A 2-L Erlenmeyer flask was charged with the above-prepared mixture of mesylates (30 g, 0.12 mol) and chloroform (800 mL). To this solution, cooled to 0° C, was added trifluoroacetic acid/water (200 mL, 1:1), and the resulting mixture was magnetically stirred at 0 °C for 90 min. The mixture was then made basic by a portionwise addition of solid potassium carbonate. Evaporation of the solvent gave a crude mixture of the desired keto mesylates. Yield: 23 g (94%).

E. A 23-g portion of the keto mesylates was refluxed with a solution of NaI (52 g) in acetone (350 mL). The reaction was continued until TLC (cyclohexane/ethyl acetate, 3:2) showed the disappearance of the starting material. The slurry was poured into water and treated with sodium thiosulfate until the solution became colorless. The solution was extracted three times with ether (3 × 150 mL), the organic extracts were dried (Na_2SO_4), and the solvents were evaporated under vacuum. The residue was column chromatographed on silica gel (cyclohexane/ethyl acetate, 9:1 as eluent) to yield 15.4 g of (±)-**3b** and 8.6 g of (±)-**3a**, which were Kugelrohr distilled at 80 °C (0.5 mmHg). R_f(cyclohexane/ethyl acetate, 3:2): 0.630 for (±)-**3a** and 0.675 for (±)-**3b**. The NMR data are reported in Table I.

Sodium Borohydride Reduction of (±)-3a. To a solution of (±)-**3a** (3.2 g, 13.4 mmol) in EtOH (50 mL), cooled at 0 °C, was added an excess of sodium borohydride. At the disappearance of the starting material, 2 N HCl was added to the mixture, the solvent was evaporated, and the residue was extracted with dichloromethane (3 × 25 mL). After the usual workup, the residue was flash chromatographed on silica gel (cyclohexane/ethyl acetate, 7:3) to yield (±)-**4** (1.84 g, 7.60 mmol) and (±)-**5** (0.9 g, 3.74 mmol) as colorless viscous oils. (±)-**4** and (±)-**5** were Kugelrohr distilled at 110 °C (0.5 mmHg). R_f(cyclohexane/ethyl acetate, 3:2): 0.529 for (±)-**4** and 0.458 for (±)-**5**.

Sodium Borohydride Reduction of (±)-3b. Following the above-reported procedure, (±)-**3b** (5.3 g, 22.1 mmol) was reduced by sodium borohydride to yield (±)-**6** (3.67 g, 15.2 mmol) and (±)-**7** (1.03 g, 4.28 mmol) as viscous oils, which were Kugelrohr distilled at 110 °C (0.5 mmHg). R_f(cyclohexane/ethyl acetate, 3:2): 0.377 for (±)-**6** and 0.401 for (±)-**7**.

20β-HSDH Reduction of (±)-3a. The following procedure is representative. A 500-mL Erlenmeyer flask was charged with iodo ketone (±)-**3a** (2.0 g, 8.32 mmol) in ethanol (25 mL), 20β-HSDH (180 units), formate dehydrogenase (50 units), NAD (0.09 mmol), 0.1 M potassium formate, and 0.05 M potassium phosphate buffer, pH 6.6 (200 mL). The mixture was gently stirred at 25 °C in the dark, and the reduction was interrupted after 24 h, at 44% conversion (GLC control). The reaction mixture was thoroughly extracted with ethyl acetate (3 × 100 mL), and the organic extracts were flash chromatographed on silica gel (eluent: cyclohexane/ethyl acetate, 4:1), to yield (+)-**3a** (0.95 g, 3.95 mmol) and (−)-**4** (0.76 g, 3.13 mmol) as colorless needles from *n*-hexane; mp 62.66 °C; molar purity 99.78.

The remaining iodo ketone (+)-**3a** (0.95 g, 3.95 mmol), dissolved in ethanol (15 mL), was resubmitted to the enzymatic reduction under the following conditions: 90 units of 20β-HSDH, 25 units of formate dehydrogenase, 0.05 mmol of NAD, 0.1 M potassium formate, and 0.05 M potassium phosphate buffer, pH 6.6 (150

(19) 20β-HSDH has been used previously for the enantioselective reduction of a series of bicycloheptenones: Leaver, J.; Gartenmann, T. C. C.; Roberts, S. M.; Turner, M. K. In *Biocatalysis in Organic Media*; Laane, C., Tramper, J., Lilly, M. D., Eds.; Elsevier: Amsterdam, 1987, pp 411–8.

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mL). The mixture was stirred at 25 °C for 36 h and then extracted with ethyl acetate (3 × 100 mL). After the usual workup the residue was flash chromatographed under the above-reported conditions to yield (+)-**3a** (0.70 g, 2.9 mmol) ($[\alpha]^{20}_D$ +28.99° (*c* 0.828, CH_2Cl_2)) and (±)-**4** (0.17 g, 0.71 mmol).

Sodium Borohydride Reduction of (+)-3a. The procedure previously described for the racemic form was applied to (+)-**3a** (0.70 g, 2.9 mmol) to yield 0.40 g (1.65 mmol) of (+)-**4** and 0.20 g of (+)-**5**.

β-HSDH Reduction of (±)-3b. A 500-mL Erlenmeyer flask was charged with (±)-**3b** (2.0 g, 8.32 mmol) in ethanol (25 mL), β-HSDH (200 units), formate dehydrogenase (70 units), NAD (0.09 mmol), 0.1 M potassium formate, and 0.05 M potassium phosphate buffer, pH 6.6 (200 mL).

The mixture was incubated for 24 h at 25 °C in the dark, before the addition of other 100 units of β-HSDH. The solution was incubated for further 48 h (conversion >95%) and then extracted with ethyl acetate (3 × 100 mL). A silica gel flash chromatography (eluent: cyclohexane/ethyl acetate, 7:3) of the residue yielded 0.90 g (3.7 mmol) of (−)-**7** as colorless needles from *n*-hexane, mp 48.93 °C; molar purity 99.80, and 0.80 g (3.3 mmol) of (+)-**6**.

Synthesis of (−)-5. A. To a magnetically stirred and ice-cooled solution of (−)-**4** (0.300 g, 1.24 mmol), triphenylphosphine (1.300 g, 4.96 mmol), and benzoic acid (0.303 g, 2.48 mmol) in dry THF (20 mL) was added dropwise a solution of DEAD (0.785 mL, 4.98 mmol) in THF (5 mL). At the disappearance of the starting material, the solvent was evaporated off and the residue was column chromatographted (eluent: cyclohexane/ethyl acetate, 9:1) to yield pure (−)-**11** (0.368 g, 86%), which was crystallized from ligroin as colorless prism, mp 68.36 °C; molar purity 99.32; R_f 0.435 (cyclohexane/ethyl acetate, 9:1); $[\alpha]^{20}_D$ −11.67° (*c* 0.934, $CHCl_3$).

B. To a solution of (−)-**11** (0.368 g) in methanol (20 mL) was added a 20% aqueous solution of potassium carbonate (20 mL). The mixture was stirred at room temperature until disappearance of the starting material (about 2 h). The organic solvent was evaporated off, and the residue was extracted with dichloromethane (4 × 25 mL). After the usual workup the residue was Kugelrohr distilled at 110 °C (0.5 mmHg) to yield 0.245 g (95%) of (−)-**5**.

Synthesis of (+)-7. Compound (+)-**6** (0.178 g, 0.735 mmol) was treated according to the above-reported procedure to give (+)-**12** (0.158 g, 62%), which was then quantitatively transformed into (+)-**7**. (+)-**12**: bp 175–180 °C (0.5 mmHg); R_f 0.38 (cyclohexane/ethyl acetate, 9:1); $[\alpha]^{20}_D$ +33.97° (*c* 0.942, $CHCl_3$).

Synthesis of (−)-6. Iodo alcohol (−)-**7** (0.125 g, 0.516 mmol) was treated according to the procedure reported for (−)-**5** to yield intermediate (−)-**13** (0.120 g, 67%), which was then quantitatively transformed into (−)-**6**. (−)-**13**: bp 170–175 °C (0.5 mmHg); R_f 0.36 (cyclohexane/ethyl acetate, 9:1); $[\alpha]^{20}_D$ −14.59° (*c* 0.514, $CHCl_3$).

Determination of the Enantiomeric Excess of Iodo Alcohols 4-7. Chiral iodo alcohols **4**–**7** and the corresponding racemic forms were converted into the (*R*)-(+)-MTPA esters,[21] and the ee values were determined by HPLC under the conditions reported in Materials and Methods.

(+)-**4**(2*R*,4*R*,5*R*): ee 96%; t_R 16.91; $[\alpha]^{30}_D$ +0.343° (*c* 1.106, $CHCl_3$).

(−)-**4**(2*S*,4*S*,5*S*): ee 96%; t_R 19.44; $[\alpha]^{30}_D$ −0.345° (*c* 1.446, $CHCl_3$) [lit.[22] $[\alpha]^{30}_D$ −0.34° (*c* 1.164, $CHCl_3$)].

(+)-**5**(2*R*,4*S*,5*R*): ee 96%; t_R 10.26; $[\alpha]^{20}_D$ +30.92° (*c* 0.835, $CHCl_3$) [lit.[10b] $[\alpha]^{22}_D$ +29.7° (*c* 2.2, EtOH)].

(−)-**5**(2*S*,4*R*,5*S*): ee 96%; t_R 8.30; $[\alpha]^{20}_D$ −30.72° (*c* 0.874, $CHCl_3$).

(+)-**6**(2*R*,4*S*,5*S*): ee >99%; t_R 19.58; $[\alpha]^{29}_D$ +40.90° (*c* 0.896, $CHCl_3$) [lit.[22] $[\alpha]^{29}_D$ +39.0° (*c* 0.508, $CHCl_3$)].

(−)-**6**(2*S*,4*R*,5*R*): ee 81%; t_R 17.60; $[\alpha]^{29}_D$ −33.12° (*c* 0.875, $CHCl_3$).

(+)-**7**(2*R*,4*R*,5*S*): ee >99%; t_R 33.05; $[\alpha]^{29}_D$ +13.43° (*c* 1.34, $CHCl_3$).

(−)-**7**(2*S*,4*S*,5*R*): ee 81%; t_R 35.16; $[\alpha]^{29}_D$ −10.64° (*c* 1.388, $CHCl_3$).

General Procedure for the Transformation of Iodo Alcohols 4-7 into the Muscarine Stereoisomers. A. A sealed metal container, charged with a solution of iodo alcohol (0.3 g, 1.2 mmol) in methanol (15 mL) and an excess dimethylamine, was heated at 80 °C overnight. The container was cooled at 0 °C, and the volatiles were evaporated under vacuum. The residue was treated with 2 N HCl (15 mL) and extracted with ether (2 × 15 mL). The aqueous layer was made basic by a portionwise addition of solid potassium carbonate and extracted with dichloromethane (3 × 15 mL). The extracts were dried (Na_2SO_4), the solvent was evaporated, and the residue was Kugelrohr distilled at 80 °C (1 mmHg). Yield: 70–75%.

B. A solution of the tertiary amine in ether was treated with an excess of methyl iodide. The precipitate was crystallized from 2-propanol. Microanalyses (C, H, and N) of **1**, **8**, **9**, and **10** agree with theoretical value ± 0.3%.

(+)-Normuscarine: $[\alpha]^{20}_D$ +11.31° (*c* 0.884, EtOH) [lit.[23] $[\alpha]^{22}_D$ +11.3° (*c* 1, EtOH)].

(−)-Normuscarine: $[\alpha]^{20}_D$ −11.39° (*c* 1.352, EtOH).

(+)-*epi*-Normuscarine: $[\alpha]^{20}_D$ +50.13° (*c* 0.764, EtOH).

(−)-*epi*-Normuscarine: $[\alpha]^{20}_D$ −50.00° (*c* 1.001, EtOH) [lit.[23] $[\alpha]^{22}_D$ −53.5° (*c* 2.5, EtOH)].

(+)-*allo*-Normuscarine: $[\alpha]^{20}_D$ +32.44° (*c* 0.524, EtOH).

(−)-*allo*-Normuscarine: $[\alpha]^{20}_D$ −38.62° (*c* 0.850, EtOH) [lit.[23] $[\alpha]^{25}_D$ −39° (*c* 2.8, EtOH).

(+)-*epiallo*-Normuscarine: $[\alpha]^{20}_D$ +16.26° (*c* 1.002, EtOH) [lit.[23] $[\alpha]_D$ +16.7° (*c* 2.3, EtOH)].

(−)-*epiallo*-Normuscarine: $[\alpha]^{20}_D$ −13.65° (*c* 0.921, EtOH).

(±)-Muscarine iodide [(±)-1]: mp 110.04 °C; molar purity 99.73% (lit.[15] 108–109 °C).

(±)-*epi*-Muscarine iodide [(±)-8]: mp 131.24 °C; molar purity 99.69% (lit.[15] 130–131 °C).

(±)-*allo*-Muscarine iodide [(±)-10]: mp 128.03 °C; molar purity 99.39% (lit.[15] 131–132 °C).

(±)-*epiallo*-Muscarine iodide [(±)-9]: mp 161.62 °C; molar purity 99.72% (lit.[15] 159–160 °C).

(+)-Muscarine iodide [(+)-1]: mp 149.22 °C molar purity 99.48; $[\alpha]^{20}_D$ +6.36° (*c* 0.346, EtOH); $[\alpha]^{20}_{578}$ +6.94°; $[\alpha]^{20}_{546}$ +7.80°; $[\alpha]^{20}_{436}$ +15.03°; $[\alpha]^{20}_{365}$ +21.09° [(lit.[10b] mp 147–148 °C; $[\alpha]^{20}_D$ +6.5° (*c* 2.2, EtOH)].

(−)-Muscarine iodide [(−)-1]: mp 147.75 °C; molar purity 99.24; $[\alpha]^{20}_D$ −5.80° (*c* 0.896, EtOH); $[\alpha]^{20}_{578}$ −5.87°; $[\alpha]^{20}_{546}$ −6.69°; $[\alpha]^{20}_{436}$ −11.64°; $[\alpha]^{20}_{365}$ −17.85°.

(+)-*epi*-Muscarine iodide [(+)-8]: mp 170.40 °C; molar purity 99.53; $[\alpha]^{20}_D$ +43.23° (*c* 0.636, EtOH); $[\alpha]^{20}_{578}$ +44.96°; $[\alpha]^{20}_{546}$ +50.94°; $[\alpha]^{20}_{436}$ +85.06°; $[\alpha]^{20}_{365}$ +131.28° [(lit.[22] 175° C; $[\alpha]^{28}_D$ +32.0° (*c* 0.550, H_2O)].

(−)-*epi*-Muscarine iodide [(−)-8]: mp 170.93 °C; molar purity 99.83; $[\alpha]^{20}_D$ −42.93° (*c* 0.750, EtOH); $[\alpha]^{20}_{578}$ −44.80°; $[\alpha]^{20}_{546}$ −50.93°; $[\alpha]^{20}_{436}$ −87.60°; $[\alpha]^{20}_{365}$ −136.13°.

(+)-*allo*-Muscarine iodide [(+)-10]: mp 130.76 °C; molar purity 99.66; $[\alpha]^{20}_D$ +31.09° (*c* 0.940, EtOH).

(−)-*allo*-Muscarine iodide [(−)-10]: mp 130.80 °C; molar purity 99.24; $[\alpha]^{20}_D$ −37.66° (*c* 0.841, EtOH) [lit[23] mp 129–30 °C; $[\alpha]^{22}_D$ −37.4° (*c* 1, H_2O)].

(+)-*epiallo*-Muscarine iodide [(+)-9]: mp 199.17 °C; molar purity 99.51; $[\alpha]^{20}_D$ 0.00° (*c* 0.700, EtOH); $[\alpha]^{20}_{578}$ +0.86°; $[\alpha]^{20}_{546}$ +1.71°; $[\alpha]^{20}_{436}$ +6.71°; $[\alpha]^{20}_{365}$ +17.86° [lit.[23] $[\alpha]_D \geq 0°$ (*c* 1, H_2O)].

(−)-*epiallo*-Muscarine iodide [(−)-9]: mp 200.68 °C; molar purity 99.24; $[\alpha]^{20}_D$ 0.00° (*c* 0.668, EtOH); $[\alpha]^{20}_{578}$ −0.78°; $[\alpha]^{20}_{546}$ −1.76°; $[\alpha]^{20}_{436}$ −6.25°; $[\alpha]^{20}_{365}$ −17.02°.

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Registry No. (+)-**1**, 24570-49-8; (−)-**1**, 79827-62-6; (±)-**1**, 2209-02-1; (+)-nor-**1**, 35119-41-6; (−)-nor-**1**, 501-38-2; (±)-**2a**, 129170-57-6; (±)-**2a** dimethyl ketal, 129170-59-8; (±)-**2a** dimethyl ketal-alcohol, 129170-61-2; (±)-**2b**, 129170-58-7; (±)-**2b** dimethyl ketal, 129170-60-1; (±)-**2b** dimethyl ketal-alcohol, 129170-62-3; (+)-**3a**, 129170-67-8; (±)-**3a**, 129064-90-0; (±)-**3a** ketal mesylate,

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129064-86-4; (±)-**3a** mesylate, 129064-88-6; (±)-**3b**, 129064-91-1; (±)-**3b** ketal mesylate, 129064-87-5; (±)-**3b** mesylate, 129064-89-7; (+)-**4**, 129170-66-7; (−)-**4**, 102735-38-6; (±)-**4**, 129170-63-4; (+)-**5**, 103664-42-2; (−)-**5**, 103664-41-1; (±)-**5**, 85316-65-0; (+)-**6**, 102735-37-5; (−)-**6**, 129170-70-3; (±)-**6**, 129170-64-5; (+)-**7**, 129170-69-0; (−)-**7**, 129170-68-9; (±)-**7**, 129170-65-6; (+)-**8**, 93226-49-4; (−)-**8**, 35119-44-9; (±)-**8**, 14400-75-0; (+)-nor-**8**, 501-37-1; (−)-nor-**8**, 34429-56-6; (+)-**9**, 5487-32-1; (−)-**9**, 129170-73-6; (±)-**9**, 104048-27-3; (+)-nor-**9**, 588-39-6; (−)-nor-**9**, 129170-72-5; (+)-**10**, 79827-61-5; (−)-**10**, 35119-38-1; (±)-**10**, 2209-03-2; (+)-nor-**10**, 129170-71-4; (−)-nor-**10**, 645-38-5; (−)-**11**, 129064-92-2; (+)-**12**, 129064-93-3; (−)-**13**, 129064-94-4.

The E1cB Mechanism in the Alkaline Hydrolysis of *N,N*-Diethyl-*P*-(3,5-dimethyl-4-hydroxyphenyl)phosphonamidic Chloride

Giorgio Cevasco* and Sergio Thea*

Istituto di Chimica Organica dell'Università e CNR Centro di Studio sui Diariloidi e loro Applicazioni, Corso Europa 26, I-16132 Genova, Italy

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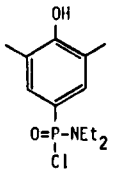
The alkaline hydrolysis of the title compound **1** proceeds via an E1cB mechanism. A tricoordinate phosphorus species intermediate **3**, or a metaphosphate-like transition state, constitutes the best hypothesis accounting for the observed kinetic results. The apparent bimolecular rate constant for the attack of hydroxide ion on the neutral 4-hydroxy-substituted chloride (k_aK_a/K_w) is more than 5 orders of magnitude larger than the true bimolecular rate constant for the attack of HO^- on the corresponding *methoxy* chloride **2** which possesses the $S_N2(P)$ mechanism. Support of the E1cB mechanism proposed for **1** comes also from activation entropy studies and by the effect of added nitrogen nucleophiles on reaction rates.

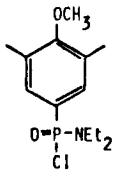
Recent work from this laboratory on acyl and sulfonyl transfer reactions has shown that aryl 4-hydroxybenzoates[1] and 4-hydroxyarenesulfonates[2] hydrolyze in moderately alkaline solutions via an E1cB mechanism involving *p*-oxoketene and sulfoquinone intermediates respectively.

In the light of the considerable biochemical significance of phosphate esters and related compounds,[3] we extended our investigations to the phosphoryl transfer reactions. Indeed, it is well known[4] that a number of such processes takes place through dissociative mechanisms, although the question of the occurrence of the monomeric metaphosphate ion (or its analogues) as an intermediate is still open.[5]

Now we report our preliminary results on the alkaline hydrolysis of *N,N*-diethyl-*P*-(3,5-dimethyl-4-hydroxyphenyl)phosphonamidic chloride (**1**) and *N,N*-diethyl-*P*-(3,5-dimethyl-4-methoxyphenyl)phosphonamidic chloride (**2**).[6]



(1)



(2)

Results and Discussion

The chloride **1** was prepared by reacting the dilithium compound obtained from 2,6-dimethyl-4-bromophenol and butyllithium with *N,N*-diethylphosphoramidic dichloride in anhydrous ether at room temperature. The reaction mixture, after the usual workup, was repeatedly chromatographed on silica gel, affording the pure product as a clear liquid. Reaction of **1** with diazomethane gave **2** as a pale yellow liquid. The identity of both **1** and **2** was assessed by ^1H NMR spectroscopy and by conversion into the corresponding 2′,4′-dinitrophenyl esters, which gave excellent elemental and spectroscopic analyses.

Results are summarized in the pH–rate profiles shown in Figure 1 (individual rate constants are given in the supplementary material). The reactions were followed spectrophotometrically by monitoring the decrease in absorbance due to the disappearance of the substrate. As it is known that in the acidic hydrolysis of phosphoroamidochloridates and phosphorodiamidic chlorides P–N bond fission may follow P–Cl bond cleavage,[7] we have checked the identity of the reaction taking place by ^1H NMR spectroscopy. We have observed that the multiplicity due to the P–N–C–H coupling of the nonequivalent methylenic hydrogen atoms of **1** disappeared upon acid hydrolysis (see the Experimental Section) whereas it was retained after alkaline hydrolysis: this fact clearly demonstrates that the P–N bond is not involved in the latter reaction.

In the pH range (7.3–14) employed, rates of hydrolysis were accurately pseudo-first-order over at least 90% of the total reaction (at pH <7 the reaction did not follow first-order kinetics any more, probably owing to the merging P–N bond fission) and depended on pH according to the rate laws 1 and 2 for compounds **1** and **2**, respectively, where K_a is the ionization costant of the phenolic group of **1**. The spectrophotometric K_a value [$K_a = (4.56 \pm 0.08) \times 10^{-9}$ M] was in good agreement with the kinetic

$$k_{obs} = [(k_o a_H/K_a) + k_a]/(1 + a_H/K_a) \quad (1)$$

$$k_{obs} = k'_o + k_b[OH^-] \quad (2)$$

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