Purities of toxins were checked on HPLC by reverse-phase Lichrosorb RP-18 (4 × 250 mm) (E. Merck) in CH_3CN/H_2O (65:35) detected by UV (254 nm). Ophiobolin A and 6-epi A were identified by NMR and MS spectra.

Toxin Bioassays. A leaf puncture bioassay test similar to that previously reported for corn and sorghum was conducted on rice cultivars S-201, Samnam, Nakdong, Aichi-asahi, ES-18, and IR-8.[4]

6-Epiophiobolin I (1): colorless oil (0.5 mg/8 L); R_f 0.50 (A) and 0.21 (B); $[\alpha]_D$ −4° (*c* 0.4, $CHCl_3$); EILRMS, *m/z* (relative intensity) 384 (21), 366 (85), 266 (35), 257 (31), 199 (27), 165 (90), 109 (100); EIHRMS, $C_{25}H_{36}O_3$ (M^+; obsd *m/z* 384.2665, calcd *m/z* 384.2666) and $C_{25}H_{34}O_2$ ($M^+ - H_2O$; obsd *m/z* 366.2559, calcd *m/z* 366.2560); ^1H NMR (250 MHz) δ 6.04 (1 H, d, *J* = 8.1 Hz, H8), 5.99 (1 H, s, H4), 5.09 (1 H, d, d, *J* = 1.2, 9.9 Hz, H18), 4.40 (1 H, d, d, d, *J* = 5.5, 8.8, 8.8 Hz, H17), 3.87 (2 H, s, H21), 3.55 (1 H, d, *J* = 6.5 Hz, H6), 3.17 (1 H, m, H2), 2.05 (3 H, s, H20), 1.70 (3 H, s, H24), 1.67 (3 H, s, H25), 1.04 (3 H, d, *J* = 7.1Hz, H23), 0.79 (3 H, s, H22).

Identification of ophiobolin I (2): R_f 0.47 (A) and 0.34 (B); EIHRMS, $C_{25}H_{36}O_3$ (M^+; obsd *m/z* 384.2665, calcd 384.2666) and $C_{25}H_{34}O_2$ ($M^+ - H_2O$; obsd *m/z* 366.2559, calcd 366.2560); ^1H NMR (250 MHz) δ 5.93 (1 H, s, H4), 5.76 (1 H, d, *J* = 4.1 Hz, H8), 5.11 (1 H, d, *J* = 8.7 Hz, H18), 4.55 (1 H, d, d, *J* = 7.3, 15.6 Hz, H17), 4.13 (1 H, d, *J* = 11.8 Hz, H21), 3.88 (1 H, d, *J* = 11.8 Hz, H21), 3.64 (1 H, d, *J* = 2.6 Hz, H6), 2.50 (1 H, d, d, *J* = 2.6, 14.6 Hz, H2), 2.19 (1 H, q, d, *J* = 7.0, 13.4 Hz, H15), 2.05 (3 H, s, H20), 1.67 (3 H, s, H24), 1.63 (3 H, s, H25), 0.99 (3 H, d, *J* = 7.0 Hz, H23), 0.96 (3 H, s, H22).

Acid Treatment of 1. A solution of 1 (1 mg) in methanol (1 mL) was added to the solution of 1 N HCl (1 mL) in methanol and then placed at room temperature for 18 h. The reaction mixture was poured into aqueous $NaHCO_3$ and then extracted with ethyl acetate. After evaporation of the organic layer under nitrogen, the residue was purified with preparative TLC ($CHCl_3$/MeOH, 14:1) to afford a single product. It was identified as ophiobolin I (2) with ^1H NMR, HRMS, TLC, and HPLC.

Ophiobolin J (3): colorless oil (20 mg/8 L); R_f 0.29 (A) and 3.31 (B); $[\alpha]_D$ +48° (*c* 1.7 $CHCl_3$); EILRMS, *m/Z* (relative intensity) 400 (6), 382 (27), 364 (10), 356 (22), 300 (24), 283 (40), 282 (31), 255 (22), 191 (33), 165 (8c), 110 (63), 109 (100); EIHRMS, $C_{25}H_{36}O_4$ (M^+; obsd *m/z* 400.2611, calcd 400.2614) and $C_{25}H_{34}O_3$ ($M^+ - H_2O$; obsd *m/z* 382.2502, calcd *m/z* 382.2509); ^1H NMR (400 MHz) δ 6.02 (1 H, t, *J* = 1.2 Hz, H4), 5.14 (1 H, d, d, d, *J* = 1.2, 1.5, 8.8 Hz, H18), 4.68 (1 H, d, d, *J* = 3.8, 6.2 Hz, H8), 4.52 (1 H, d, *J* = 13.9 Hz, H21), 4.51 (1 H, d, d, *J* = 8.8, 15.6 Hz, H17), 4.41 (1 H, d, *J* = 13.9 Hz, H21), 4.04 (1 H, br d, *J* = 11.2 Hz, H2), 2.10 (3 H, d, *J* = 0.5 Hz, H20), 1.70 (3 H, d, *J* = 1.2 Hz, H24), 1.64 (3 H, d, *J* = 1.2 Hz, H25), 1.16 (3 H, s, H22), 1.03 (3 H, d, *J* = 6.8 Hz, H23); ^{13}C NMR (125 MHz) δ 202.3 (s, C5), 177.5 (s, C3), 149.1 (s, C7), 138.5 (s, C6), 134.5 (s, C19), 131.2 (d, C4), 126.8 (d, C18), 95.9 (s, C14), 74.8 (d, C8), 71.6 (d, C17), 62.4 (t, C21), 52.4 (d, C10), 49.8 (t, C1), 44.3 (d, C2), 42.3 (t, C16), 41.6 (s, C11), 41.3 (t, C12), 36.0 (d, C15), 30.8 (t, C9), 30.6 (t, C13), 25.8 (q, C24), 21.7 (q, C22), 18.1 (q, C25), 17.3 (q, C20), 16.2 (q, C23).

8-Deoxyophiobolin J (4): colorless needle crystal (1.2 mg/8 L); mp 138–140 °C; R_f 0.56 (A) and 0.49 (B); $[\alpha]_D$ +8° (*c* 0.15, $CHCl_3$); EILRMS, *m/z* (relative intensity) 384 (18), 366 (27), 302 (15), 284 (30), 266 (19), 201 (39), 199 (30), 165 (83), 109 (100); EIHRMS, $C_{25}H_{36}O_3$ (M^+; obsd *m/z* 384.2663, calcd *m/z* 384.2666) and $C_{25}H_{34}O_4$ ($M^+ - H_2O$; obsd *m/z* 366.2558, calcd *m/z* 366.2560); ^1H NMR (250 MHz) δ 6.00 (1 H, s, H4), 5.65 (1 H, d, *J* = 9.0 Hz, H18), 4.52 (1 H, d, d, *J* = 7.4, 15.7 Hz, H17), 4.36 (1 H, d, *J* = 13.0 Hz, H21), 4.31 (1 H, d, *J* = 13.0 Hz, H21), 3.17 (1 H, d, *J* = 12.0 Hz, H2), 2.07 (3 H, s, H20), 1.66 (3 H, s, H24), 1.60 (3 H, s, H25), 1.06 (3 H, s, H22), 0.99 (3 H, d, *J* = 6.9 Hz, H23).

Bis(*p*-bromobenzoate) of 3: R_f 0.75 ($CHCl_3$/MeOH, 50:1); ^1H NMR (250 MHz) δ 7.91 (1 H, d, *J* = 8.3 Hz, Ar H), 7.80 (1 H, d, *J* = 8.3 Hz, Ar H), 7.56 (1 H, d, *J* = 8.3 Hz, Ar H), 7.51 (1 H, *J* = 8.3 Hz, Ar H), 6.00 (1 H, s, H4), 5.95 (1 H, d, *J* = 5.8 Hz, H8), 5.73 (1 H, d, *J* = 13.4 Hz, H21), 5.65 (1 H, d, *J* = 13.4 Hz, H21), 5.04 (1 H, d, *J* = 8.5 Hz, H18), 4.51 (1 H, d, d, *J* = 7.2, 15.0 Hz, H17), 3.74 (1 H, d, *J* = 10.9 Hz, H2), 2.02 (3 H, s, H20), 1.57 (3 H, s, H24), 1.53 (3 H, s, H25), 1.19 (3 H, s, H22), 1.02 (3 H, d, *J* = 6.8 Hz, H23).

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Stereochemistry of the *N*-Methyl Group in Salts of Tropane Alkaloids

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^1H and ^{13}C NMR slow exchange limit spectra of atropine sulfate/mesylate, homatropine hydrobromide, and benztropine mesylate solutions concur in showing that, at equilibrium, the equatorial:axial *N*-CH_3 diastereomeric mixture was ca. 7:1 (D_2O) and ca. 18:1 (CD_2Cl_2). A similar preponderance of equatorial *N*-CH_3 stereochemistry was observed for cocaine salts in both solvents (only equatorial isomer noted in D_2O ^{13}C NMR spectrum, while ca. 18:1 equatorial:axial ratio found in CD_2Cl_2). The *N*-CH_3 orientation in scopolamine hydrobromide was strikingly solvent sensitive and underwent a reversal from an equatorial:axial *N*-CH_3 ratio of ca. 1:18 (D_2O) to ca. 18:1 (CD_2Cl_2). Solid-state CP-MAS ^{13}C NMR spectra of crystalline equatorial *N*-CH_3 (8*s*)-atropine sulfate and axial *N*-CH_3 (9*r*)-scopolamine hydrobromide confirmed the stereochemical assignments for major and minor diastereomers in solution.

Introduction

Atropine [*dl*-hyoscyamine[3] (1)], scopolamine [*l*-hyoscine[4] (2)], and homatropine[5] (*dl*-3) are antimuscarinic (or atropinic) drugs that inhibit the action of acetylcholine on structures innervated by postganglionic parasympathetic nerves.[6] *l*-Hyoscyamine (1) and *l*-hyoscine (2) are natural products isolated from belladonna plants [*atropa bella-*

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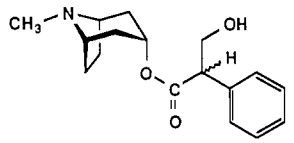
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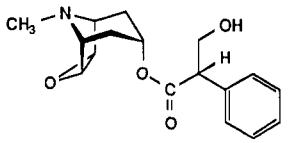
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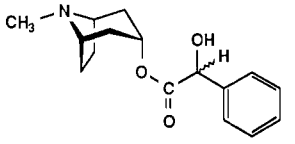
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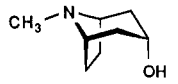
donna (nightshade) and *hyoscyamus niger* (henbane), respectively] and consist of (C_α-*S*)-tropic acid esters of the respective 3-endo-substituted amino alcohols, tropine (**4**) and scopine (**5**).[6,7] Homatropine (**3**) is a synthetic ace-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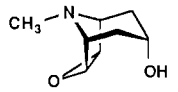
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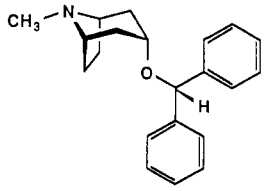
tylcholine antagonist. Benztropine[8] (**6**), a synthetic tropine ester, is a centrally acting muscle relaxant used in the treatment of Parkinson's disease.[9] *l*-Cocaine[10] [(1*R*,2*R*,3*S*,5*S*)[11]-(**7**)] is a coca alkaloid isolated from the *erythroxylon coca* plant[12] and was the first local anesthetic to be discovered.[13]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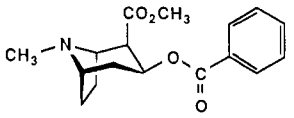
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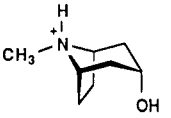
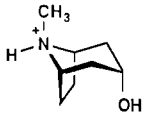
Tropane alkaloids readily undergo epimerization in solution via nitrogen inversion and result in the formation of (*N*-*r*,*s* or -*R*,*S*) *N*-CH_3 diastereomers. A similar diastereomerization process for the corresponding salts proceeds via a prototropic shift at the labile chirotopic (or achirotopic) stereogenic[14] nitrogen atom and involves nitrogen inversion in the free base. Increasing NMR time scale lifetimes of the two equilibrium species enables the recording of spectra for both diastereomers at the slow exchange limit (SEL) for isomer interconversion. A very early ^{1}H NMR investigation of this SEL phenomenon was performed at 40 MHz by Closs[15] in 1959: an acidic aqueous solution (pH 1) of **4** afforded a ca. 16:1 preference of equatorial *N*-CH_3 (8*s*)-tropine hydrochloride (**8e**) over the axial (8*r*)-diastereomer (**8a**) at ambient temperature.[15] Two unequally populated doublets [both $^3J(NH\text{–}CH_3)$ ca. 5 Hz (H_2O, pH 1); two $^+NDCH_3$ singlets in acidic D_2O (pD


8e


8a

1)] became one weighted-average broadened singlet at pH 6 (H_2O) via chemical exchange decoupling.[15] Closs also reported a ca. 20:1 **e**:**a** mixture of **1a,e·DCl** atropine deuteriochloride *N*-CH_3 diastereomers under the same acidic aqueous conditions.[15] These two atropine 8*r*,*s* diastereomers were also observed in the acidic CD_3OD ^{13}C NMR spectrum.[16] The use of reduced temperature to increase the lifetimes of two equilibrium free-base species was demonstrated by Schneider and Stürm[17] using ^{13}C NMR at −70 °C [ca. 20:1 preference of the equatorial *N*-CH_3 8*s* diastereomer of tropine free base (**4e**)]. Configurational assignments were based on the γ-gauche effect[18] due to *N*-CH_3.

Spectral parameters at the ^{1}H NMR fast exchange limit (FEL) for interconversion (in this case dissolution in D_2O without addition of acid) relate to a "time-averaged" structure that does not exist chemically. More caution must be taken in drawing stereochemical conclusions from FEL data versus that obtained from studies at the SEL. In addition, based upon the magnitude of chemical shift differences between the particular exchanging nuclei, heteronuclear NMR studies can result in spectra having different kinetic exchange rate regimes. Thus, averaged ^{1}H NMR FEL spectral parameters were noted for atropine sulfate (1·0.5H_2SO_4) recorded in D_2O without addition of excess acid,[15] while the ^{13}C NMR spectrum afforded two sets of externally diastereotopic SEL carbon signals (i.e., the direct observation of two diastereomers).[19] Wenkert et al.[20] performed the first ^{13}C NMR study on tropane alkaloid free bases [equatorial *N*-CH_3 preference was noted using FEL chemical shift arguments for nortropane, tropane, and C(3)-oxygenated derivatives]. Numerous ^{1}H and ^{13}C NMR investigations have been performed on the free bases[20–32] and salts[15,16,19,27,30,31,33,34] of the above-mentioned

(7) Preparations of the deadly belladonna plants have been used therapeutically and as poisons for many centuries. The *atropa belladonna* shrub was named after Atropos (the oldest of the three Fates), known to be the one who cuts the Thread of Life (ref 6).

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Table I. Aliphatic ^1H NMR Slow Exchange Limit Chemical Shift Values for the Equatorial N-CH_3 Diastereomers of Atropine Sulfate (1e•0.5H_2SO_4), Atropine Mesylate (1e•CH_3SO_2OH), Scopolamine Hydrobromide (2e•HBr), Homatropine Hydrobromide (3e•HBr), Benztropine Mesylate (6e•CH_3SO_2OH), and Cocaine Hydrochloride (7e•HCl)[a]

δ_H	1e·0.5H_2SO_4	1e·CH_3SO_2OH	2e·HBr	3e·HBr	6e·CH_3SO_2OH	7e·HCl
H(1)	3.51	3.57	3.68	3.49	3.73	4.27
H(5)	3.64	3.71	3.82	3.67	3.73	4.17
H(21)	2.88	2.62	3.21	2.98	2.56	*b*
H(41)	2.96	2.71	3.31	3.11	2.56	3.07
H(22)	1.75	1.80	1.77	1.72	ca. 2.1	3.50
H(42)	1.96	2.01	1.99	ca. 2.0	ca. 2.1	2.25
H(31), H(32)	5.14[c]	5.10[c]	5.18[c]	5.16[c]	3.76[c]	5.46[d]
H(61)		1.51	2.76	1.02	ca. 2.5	ca. 2.1
H(71)		2.14	3.62	ca. 1.7	ca. 2.5	ca. 2.1
H(62)		ca. 2.0	*b*	ca. 2.0	ca. 2.1	ca. 2.4
H(72)		ca. 1.8	*b*	ca. 2.0	ca. 2.1	ca. 2.4
N-CH_3(eq)	2.60	2.67	2.87	2.59	2.73	3.01
[N-CH_3(ax)][e]	[2.73]	[2.80]	[2.83]	[2.71]	[2.80]	[2.93]
N-*H*	12.07	10.46	11.84	10.95	10.64	10.53
H(α)	3.79	3.80	3.79	5.14[f]	5.44[g]	*b*
H(β)	3.84	3.84	3.83	*b*	*b*	*b*
H(β')	4.17	4.16	4.16	*b*	*b*	*b*
O-CH_3	*b*	*b*	*b*	*b*	*b*	3.76[h]
S-CH_3	*b*	2.74	*b*	*b*	2.68	*b*

[a] Ppm downfield from tetramethylsilane, 400 MHz [with the exception of 1e·0.5H_2SO_4 (200 MHz)], CD_2Cl_2, 294 K. [b] Not applicable. [c] H(31). [d] H(32). [e] δ-value for axial N-CH_3 in (N-*r*,*R*) diastereomer given in square brackets. [f] 5.13 ppm for **3a**·HBr. [g] Benzhydryl-*H*; 5.46 ppm for **6a**·CH_3SO_2OH. [h] 3.73 ppm for **7a**·HCl.

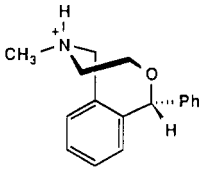
Table II. Aliphatic ^1H NMR Slow Exchange Limit Homonuclear Coupling Constants (Hz) for the Equatorial N-CH_3 Diastereomers of Atropine Mesylate (1e•CH_3SO_2OH), Scopolamine Hydrobromide (2e•HBr), Homatropine Hydrobromide (3e•HBr), Benztropine Mesylate (6e•CH_3SO_2OH), and Cocaine Hydrochloride (7e•HCl)[a]

J(H–H)	1e·CH_3-SO_2OH	2e·HBr	3e·HBr	6e·CH_3-SO_2OH	7e·HCl
1–21	3.4 (3)	3.3 (6)	4.4 (1)		*b*
5–41	3.2 (3)	3.5 (2)	4.0 (5)		2.5 (3)
1–22	3.6 (4)	3.2 (5)	3.2 (6)		2.2 (2)
5–42	3.4 (2)	3.2 (5)			3.1 (4)
1–71	<1	<1			
5–61	<1	<1	<1		
1–72	7.2	*b*	6.7 (5)		7.6 (3)
5–62	6.9	*b*			
21–22	−16.1 (3)	−16.4 (4)	−16.4 (4)	−14.6	*b*
41–42	−14.9 (8)	−16.2 (3)	−15.9 (4)	−14.6	−14.3 (3)
21–31	4 (1)	4.9 (3)	4.6 (3)	4.5	*b*
(31, 32)–41	4 (1)[c]	5.1 (3)[c]	4.3 (6)[c]	4.5[c]	12.4 (4)[d]
22–(31, 32)	<1[e]	<1[e]	<1[e]	<1[e]	7.1 (2)[f]
(31, 32)–42	<1[g]	<1[g]	<1[g]	<1[g]	6.2 (2)[h]
N*H*-CH_3-(eq)	5.0	5.1 (2)	5.2	5.0	5.1 (1)
[N*H*-CH_3-(ax)][i]	[5.0]	[5.2]	[5.5]	[5.0]	[5.2 (1)]
61–62	−14.0 (4)	*b*	−14.2 (1)		
71–72	−14.2 (4)	*b*			
61–71	9.9 (3)	3.0 (1)	9.2 (2)		
61–72	4.3 (4)	*b*	5.2 (2)		
62–71	4.5 (4)	*b*			
21–72			1.7 (4)[j]		2.7 (5)[j]
22–42					2.8 (2)[j]

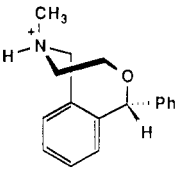
[a] Hz, standard deviation given in parentheses, 400 MHz, CD_2Cl_2, 294 K. [b] Not applicable. [c] J(32–41). [d] J(31–41). [e] J(22–32). [f] J-(22–31). [g] J(32–42). [h] J(31–42). [i] J value for axial N*H*–CH_3 in (N-*r*,*R*) diastereomer given in square brackets. [j] Sign not determined.

alkaloids. Unfortunately, some of these reports suffer from misassignments of chemical shifts, e.g., the N-CH_3 chemical shift for scopolamine hydrobromide (2·HBr) [reported as 53.42,[33] 34.31,[31] or 25.72[19] ppm (D_2O)].

We have recently shown that dissolution of equatorial N-CH_3 crystalline nefopam hydrochloride (**9e**·HCl) analgesic afforded a mixture of two N-CH_3 diastereomers (**9a,e**·HCl) (e:a ratio ca. 2:3 in ambient temperature CD_2Cl_2 or ca. 1:1 in acidic D_2O).[35]



9e



9a

As a continuation of the above study, we report the N-CH_3 stereochemistry of tropane cations in both D_2O and CD_2Cl_2 solutions. In addition, solid-state CP-MAS ^{13}C NMR spectra of crystalline scopolamine hydrobromide (**2a**·HBr) [axial N-CH_3 (X-ray crystallography)] and atropine sulfate (1e·0.5H_2SO_4) (equatorial N-CH_3) are presented for definitive assignment of the corresponding N-CH_3 signals in solution.

Results and Discussion

Tables I–IV list ^1H and ^{13}C NMR SEL spectral parameters for atropine sulfate (1·0.5H_2SO_4), atropine mesylate (1·CH_3SO_2OH), scopolamine hydrobromide (2·HBr), homatropine hydrobromide (3·HBr), benztropine mesylate (6·CH_3SO_2OH), and cocaine hydrochloride (7·HCl) (N-*r*,*s* or *R*,*S*)-N-CH_3 diastereomeric mixtures. Atropine sulfate (1·0.5H_2SO_4), homatropine hydrobromide (3·HBr), and benztropine mesylate (6·CH_3SO_2OH) all gave SEL D_2O ^{13}C NMR spectra showing two N-CH_3 diastereomers (ca. 7:1 ratio). Scopolamine hydrobromide (2·HBr) gave a *dynamic* spectrum at 295 K showing decidedly broadened signals for C(1,5), C(2,4), and especially N-CH_3 (see numbering diagram **10**). Two drops of trifluoroacetic acid (TFA) added to the 3-mL ^{13}C NMR sample (ca. 0.15 M) dramatically sharpened all spectral lines and now enabled the SEL observation of two species (ca. 1:18). This behavior is in accord with the known higher acidity for protonated scopolamine cations (pK_a = 7.55) vis-ā-vis that for protonated atropine and homatropine cations (pK_a =

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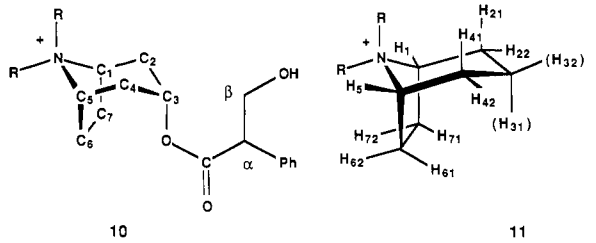

10 11

9.25 and 9.7, respectively).[36] The pH values of 0.15 M aqueous solutions of scopolamine hydrobromide (2·HBr), atropine sulfate (1·0.5H_2SO_4), and homatropine hydrobromide (3·HBr) were measured as: 5.10, 7.00, and 6.65, respectively. This suggests that TFA attenuation of free-base concentration slowed the relatively fast rate of nitrogen inversion in aqueous solutions of scopolamine salts. Avdovitch and Neville[31] also noted broadened lines in the ^{13}C NMR spectrum (D_2O) of tropine free base (4) that dramatically sharpened upon addition of "a few drops" of TFA. However, they ascribed this broadening to intermolecular H-bonding relaxation phenomena within associated species (which are then subsequently destroyed upon addition of TFA).[31] The ^{13}C NMR spectrum of cocaine hydrochloride (7·HCl) (0.05 M in D_2O), with narrow lines at 298 K similar to that observed in the nondynamic SEL spectra of 1,3,6·HX, showed the discernible presence of only one N-CH_3 diastereomer. Addition of TFA to the 3-mL NMR solution of 7·HCl (pK_a = 8.5) did not alter the ^{13}C NMR spectrum.

Atropine sulfate (1·0.5H_2SO_4) was found to be very poorly soluble in CD_2Cl_2. This precluded the measurement of $^{13}C\{^1H\}$ NMR spectra for this sample. A marked improvement in CD_2Cl_2 solubility was noted when the divalent sulfate anion was replaced by a monovalent mesylate anion. Similar higher CD_2Cl_2 solubilities were seen for benztropine mesylate (6·CH_3SO_2OH), while intermediate degrees of solubility were found for salts of 2, 3, and 7 containing bromide or chloride anions.

Major and minor species (ca. 18:1) were observed in both the ^1H and ^{13}C NMR spectra for *all* five alkaloid salts of 1–3, and 6, 7 in CD_2Cl_2. Both species are protonated in this medium as evidenced by major,minor N-CH_3 doublet multiplicity [$^3J(NH-CH_3)$ ca. 5 Hz]. Aside from CH_3 resonances, other signals for the minor species were not clearly ascertained in the ^1H NMR spectra. Enantiotopic ^1H and ^{13}C nuclei within the tropine and scopine moieties were rendered internally diastereotopic[37] and thus anisochronous by the presence of the stereogenic chirotopic[14] C(α) atoms in the tropic and mandelic acid moieties. This diastereotopicity found for the amine salts of 1–3 at the ^1H SEL (and at the ^{13}C SEL[16,19,31,33]) was also noted at the ^1H FEL for the salts of 1 and 2[19] and for the free bases 1–3.[21,23,25,28,29,31,32]

Carbon-13 NMR Spectral Parameters. ^{13}C NMR assignments were made by using the single-frequency offset resonance decoupling (SFORD) technique (50 MHz, D_2O) or the DEPT[38] pulse sequence (100 MHz, CD_2Cl_2). With the exception of the D_2O solution of *l*-cocaine hydrochloride (7·HCl), all the tropane cations (irrespective of the solvent used) gave spectra having two unequal-magnitude N-CH_3 signals [33 (1) and 41 (3) ppm]. N-CH_3 orientations giving rise to the two above-mentioned resonances were unequivocally shown to be axial and equatorial, respectively, by means of solid-state CP-MAS ^{13}C NMR. Crystalline (9*r*,C_α-*S*)-hyoscine hydrobromide [(−)-scopolamine hydrobromide (2a·HBr)][39a] and (8*s*,C_α-*S*)-hyoscyamine hydrobromide [(−) enantiomer of atropine hydrobromide (1e·HBr)][39b,40] have axial and equatorial N-CH_3 groups, respectively, as determined by single-crystal X-ray diffraction analysis. Equatorial N-CH_3 orientations are usually the rule in tropane molecules due to unfavorable cis-1,3 nonbonded interactions between axial N-CH_3 and piperidine-ring axial protons: e.g., X-ray crystal structures of (8*s*)-tropine hydrobromide,[41]

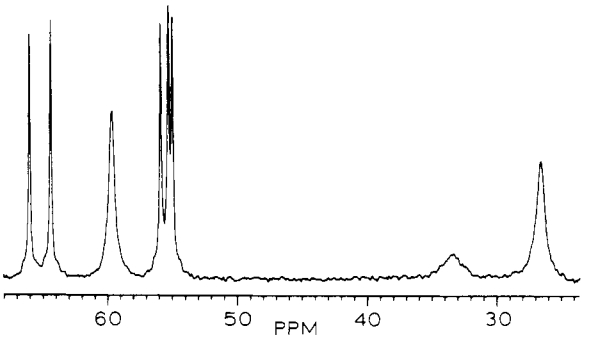

Figure 1. $^{13}C\{^1H\}$ NMR (50.3 MHz, 295 K) aliphatic region of scopolamine hydrobromide (2·HBr) showing dynamic exchange broadening in D_2O.

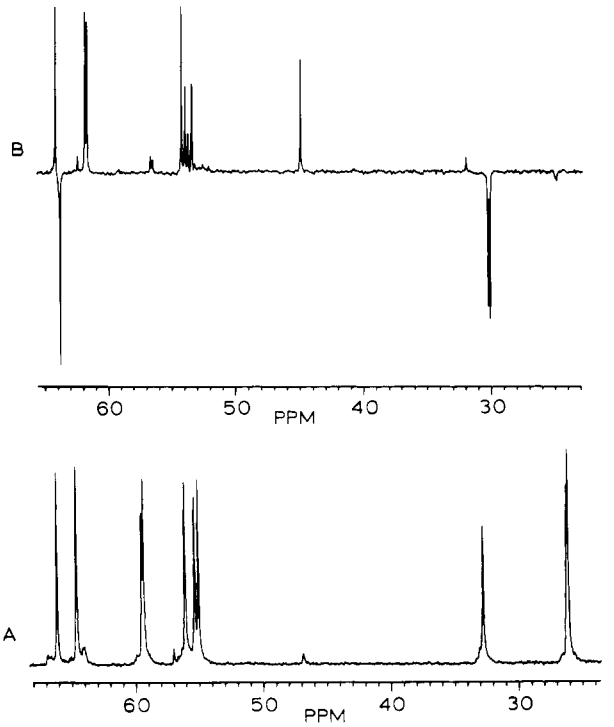

Figure 2. Trace A: $^{13}C\{^1H\}$ NMR (50.3 MHz, 298 K) aliphatic region of (9*r*,*s*)-scopolamine hydrobromide (2a,e·HBr) N-CH_3 diastereomeric mixture, a:e ratio ca. 18:1 (0.15 M solution of 2a·HBr in 3 mL of D_2O and 2 drops of trifluoroacetic acid). Trace B: $^{13}C\{^1H\}$ NMR (100.6 MHz, DEPT pulse sequence, 135° pulse width, 294 K) aliphatic region of (9*r*,*s*)-scopolamine hydrobromide (2a,e·HBr) N-CH_3 diastereomeric mixture, a:e ratio ca. 1:18 (in CD_2Cl_2).

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Table III. $^{13}C\{^1H\}$ NMR Slow Exchange Limit Chemical Shift Values for Equatorial (e) and Axial (a) N-CH_3 Diastereomeric Mixtures of Atropine Sulfate (1a,e•0.5H_2SO_4), Atropine Mesylate (1a,e•CH_3SO_2OH), and Scopolamine Hydrobromide (2a,e•HBr)[a]

	1·0.5H_2SO_4/1·CH_3SO_2OH[b]					2·HBr[c]				
	eq N-methyl			ax N-methyl		eq N-methyl		ax N-methyl		
δ_C	D_2O	CD_2Cl_2	solid	D_2O	CD_2Cl_2	D_2O	CD_2Cl_2	D_2O	CD_2Cl_2	solid
C(1)	64.59	62.29	61.76	60.93	58.31	63.94	61.81	59.34	56.58	56.69
C(5)	64.71	62.39	61.76	61.03	58.41	64.09	61.96	59.48	56.77	57.81
C(2)	36.75	34.53	35.00	30.13	28.49		30.17	26.10	24.89	23.95
C(4)	36.89	34.70	35.47	30.13	28.61		30.34	26.22	25.01	24.71
C(3)	68.07	65.07	68.57		64.21	66.73	64.30	66.05	62.53	64.82
C(6)	25.43	23.59	24.17	27.45	25.44		53.49	55.01	52.15	53.09
C(7)	25.67	24.05	26.07	27.67	25.80		54.01	55.30	52.62	53.09
N-CH_3	41.11	39.27	39.56	33.95	31.95	46.86	45.01	32.74	32.00	33.19
C(α)	56.21	54.50	56.76	56.21	54.50	56.06	54.32	56.06	54.32	53.09
C(β)	64.71	63.97	65.53	64.71	64.13	64.57	63.95	64.57	64.06	61.38
C=O	175.53	*d*	172.81	175.53	*d*	174.86	*d*	174.86	*d*	172.07
C_{ipso}	137.83	*d*	138.01	137.83	*d*	138.16	*d*	138.16	*d*	139.00
C_o	131.62	129.23	132.89	131.62	129.23	131.66	129.27	131.66	129.27	133.78
C_m	130.74	128.45	129.63	130.74	128.45	130.85	128.29	130.85	128.29	132.38
C_p	130.63	128.12	127.40	130.63	128.12	130.69	128.29	130.69	128.29	128.29
S-CH_3	*e*	39.42[f]	*e*	*e*	39.42[f]	*e*	*e*	*e*	*e*	*e*

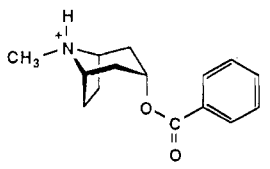
[a] Ppm downfield from tetramethylsilane (except for aqueous solutions, where 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt was used as internal reference); hexamethylbenzene used as secondary reference for solid-state spectra. Spectra recorded in CD_2Cl_2 were measured at 100 MHz (294 K, DEPT pulse sequence; those in D_2O (294 K) and the solid state (301 K, TOSS pulse sequence) were measured at 50 MHz. Assignments within a diastereotopic pair are arbitrary. [b] **1e,a**·0.5H_2SO_4, **1e,a**·CH_3SO_2OH, and **1e**·0.5H_2SO_4 recorded in D_2O (**e:a** ratio ca. 7:1), CD_2Cl_2 (**e:a** ratio ca. 18:1), and the solid state, respectively. [c] **2e,a**·HBr recorded in D_2O (**e:a** ratio ca. 1:18) and CD_2Cl_2 (**e:a** ratio ca. 18:1); **2a**·HBr recorded in the solid state. [d] Quaternary carbons C_{ipso} and C=O not observed in DEPT. [e] Not applicable. [f] S-CH_3 value may be interchanged with the equatorial N-CH_3 value in CD_2Cl_2 solution.

Table IV. $^{13}C\{^1H\}$ NMR Slow Exchange Limit Chemical Shift Values for Equatorial (e) and Axial (a) N-CH_3 Diastereomeric Mixtures of Homatropine Hydrobromide (3a,e•HBr), Benztropine Mesylate (6a,e•CH_3SO_2OH), and Cocaine Hydrochloride (7a,e•HCl)[a]

	3e·HBr		3a·HBr		6e·CH_3SO_2OH		6a·$CHSO_2OH$		7e·HCl		7a·HCl
δ_C	D_2O	CD_2Cl_2	D_2O	CD_2Cl_2	D_2O	CD_2Cl_2	D_2O	CD_2Cl_2	D_2O	CD_2Cl_2	D_2O
C(1)	64.59	62.29	60.51	58.07	64.70	63.03	60.97	58.95	66.48	63.94	61.74
C(5)	64.76	62.43	60.67	58.20	64.70	63.03	60.97	58.95	65.64	63.58	59.84
C(2)	36.87	34.53	29.73	28.92	36.50	34.81	29.67	28.78	48.69	47.34	45.64
C(4)	36.92	34.53	29.78	28.92	36.50	34.81	29.67	28.78	35.13	33.36	30.05
C(3)	68.57	66.98		65.91	68.74	67.25	68.25	66.52	66.85	64.03	63.29
C(6)	25.23	23.80	27.27	25.35	25.61	24.66	27.64	26.42	26.13	25.55	27.62
C(7)	25.69	24.45	27.67	25.98	25.61	24.66	27.64	26.42	25.08	23.27	27.44
N-CH_3	41.16	39.38	34.05	31.97	40.64	39.55	33.41	32.02	41.43	40.48	33.06
C(α)	75.57	73.48	75.57	73.48	82.81[b]	81.74[b]	82.62[b]	81.74[b]	*d*	*d*	*d*
C=O	175.44	*c*	175.44	*c*	*d*	*d*	*d*	*d*	169.51	165.89	165.89
C=O(OMe)	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	175.71	170.08	170.08
C_{ipso}	140.10	*c*	140.10	*c*	144.36	142.48	144.36	142.48	130.95	129.50	129.50
C_o[e]	129.62	127.06	129.62	127.06	130.63	128.83	130.63	128.83	131.48	128.89	128.89
C_m[e]	131.70	129.14	131.70	129.14	128.84	126.91	128.84	126.91	131.97	129.94	129.94
C_p	131.70	129.14	131.70	129.14	129.62	127.89	129.62	127.89	136.90	133.94	133.94
S-CH_3	*d*	*d*	*d*	*d*	40.71[f]	39.46[f]	40.71[f]	39.46[f]	*d*	*d*	*d*
O-CH_3	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	55.83	53.42	52.72

[a] Ppm downfield from tetramethylsilane (except for aqueous solutions, where 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt was used as internal reference). Spectra recorded in CD_2Cl_2 were measured at 100 MHz (294 K, DEPT pulse sequence); those in D_2O were measured at 50 MHz (294 K). Assignments within a diastereotopic pair or C(6, 7) in **7e,a**·HCl are arbitrary. **3a,e**·HBr, **6a,e**·CH_3SO_2OH, and **7e,a**·HCl recorded in D_2O (both **3e:a** and **6e:a** ratios ca. 7:1; **7e**·HCl epimer >95%) and in CD_2Cl_2 (all **e:a** ratios ca. 18:1). [b] Benzhydryl-C. [c] Quaternary carbons C_{ipso} and C=O not observed in DEPT. [d] Not applicable. [e] Assignments of ortho and meta carbons may be interchanged. [f] S-CH_3 value may be interchanged with the equatorial N-CH_3 value in CD_2Cl_2 solution.

(1R,2R,3S,5S,8S)-cocaine hydrochloride,[42] (8s,C$_\alpha$-S)-hyoscyamine hydrobromide,[39b,40] (8s)-pseudotropine free base,[43] (8s)-O-benzoyltropine hydrochloride (**12e**),[44]



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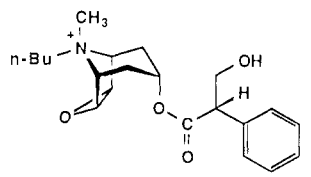

13a

(8s)-O-benzoylpseudotropine hydrochloride,[45] (8s)-benztropine mesylate,[46] and (3r,8s)-3-bromotropane hydrobromide[47] [(9r,C$_\alpha$-S)-hyoscine hydrobromide[37] and related compounds: (9s,C$_\alpha$-S)-N-butylhyoscinium bromide (**13a**),[48] (9s,C$_\alpha$-S)-scopolamine N-oxide hydrobromide[49] are ex-

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ceptions].

The solid-state CP-MAS ^{13}C NMR chemical shift for the axial N-CH_3 in crystalline scopolamine hydrobromide (**2a**·HBr) was 33.19 ppm. The corresponding absorbance for N-CH_3 in crystalline atropine sulfate (**1e**·$0.5H_2SO_4$) was 39.56 ppm and was in complete accord with an equatorial N-CH_3 orientation. The N-CH_3 signal in the CP-MAS ^{13}C NMR spectrum was confirmed by a dipolar dephasing experiment[50] based on less efficient solid-state relaxation for methyl carbons (via-â-vis methylene and methine carbons). After a suitable delay period was introduced prior to FID acquisition, N-CH_3 and $C_{quaternary}$ magnetization was still noted in the spectrum. The solid-state N-CH_3 absorbances of 33.19 and 39.56 ppm were correlated with the 33 (1) and 41 (3) ppm signals for N-CH_3 in D_2O or CD_2Cl_2 solution.

In this manner, configurational descriptors were assigned to the two N-CH_3 diastereomers that gave rise to the set of unequal intensity signals. Thus, the ^{13}C resonances in the equatorial N-CH_3 (8s)-tropane salts were found to be δ 41 (3) (CH_3), 25 (1) and 35 (2) (CH_2), and 63 (1) and 67 (2) [CH, two 63 (1) ppm signals usually seen]. Similarly, the corresponding absorbances for the axial N-CH_3 8r-epimeric salts in this study were observed as δ 33 (1) [CH_3], 27 (1) and 28 (2) (CH_2), and 59 (2) and 65 (2) [CH, two 59 (2) ppm signals usually seen]. CH_2 assignments can be made by comparison of the C(2) signals in piperidines and pyrrolidines[20] or by consideration of the spectral parameters of cocaine hydrochloride (**7**·HCl) (only one CH_2 in the piperidine ring). The 25 (1) and 35 (2) ppm CH_2 signals arise from C(6,7) and C(2,4) nuclei, respecively, in the equatorial N-CH_3 8S diastereomer, while the 27 (1) and 28 (2) ppm signals come from the corresponding carbons in the axial N-CH_3 8R epimer. The most deshielded methine carbon is C(3) for each diastereomer, in accord with both the expected adjacent oxygen electronegativity effect and the similar slightly higher field chemical shifts for the diastereotopic C(1,5) pair. Tropic acid C(α),C(β) signals [55 (1) and 64 (1) ppm, respectively] were assigned according to known values.[32,33] Differential assignments were not made within a diastereotopic pair [e.g., C(1,5)] or within the **7**-HCl heterotopic C(6,7) pair.

SEL ^{13}C NMR spectral parameters of only one diastereomeric pair of equatorial,axial N-CH_3 tropane cations were reported in the literature [atropine sulfate (**1a,e**·$0.5H_2SO_4$) in D_2O[19] and atropine hydrobromide (**1a,e**·HBr) in acidic CD_3OD[16]]. ^{13}C NMR data on single species for atropine sulfate (**1**·$0.5H_2SO_4$),[31,33] scopolamine hydrobromide (**2**·HBr),[19,33] scopolamine hydrochloride (**2**·HCl),[31] and cocaine hydrochloride (**7**·HCl)[31] in D_2O solution are also known. The scopolamine hydrobromide (**2**·HBr) N-CH_3 signal has been erroneously reported as being at 53.42 ppm[33] [actually one of the C(6,7) pair] and also at 25.72 ppm[19] [one of the C(2,4) pair]. Moreover, Simeral and Maciel[33] assigned the 53.75 ppm signal [one of the C(6,7) pair] as that of C(1,5), while assigning the 58.26 and 58.05 ppm [C(1,5)] signals as those of C(6,7). The very broadened low-intensity N-CH_3 signal ($W_{1/2}$ = 55 Hz) in the **2**·HBr spectrum expresses its dynamic nature in D_2O and probably lies at the root of this confusion. Our 32.74 ppm value [from a narrow axial N-CH_3 resonance, sharpened by addition of two drops of TFA (vide ante)] is comparable with the 34.31 ppm[31] value reported by Avdovitch and Neville. While these authors added TFA to an aqueous solution of tropine free base (**4**) for spectral line sharpening (vide ante), no mention was made of a similar treatment for the scopolamine salt. When systematic differences arising from different reference standards are taken into account, our chemical shift values are in good agreement with those in the literature, and with the exception of the discrepancies noted above, our assignments of **1**·$0.5H_2SO_4$, **2**·HBr, and **7**·HCl ^{13}C NMR resonances are in accord with corrections made by Pook et al.,[16] Feeney et al.,[19] Baker et al.,[34] and Avdovitch and Neville.[31]

The five alkaloid salts of this study all show typical upfield shifts for piperidine-ring carbons β, γ, and δ to axial N-CH_3 vis-â-vis those for corresponding nuclei in the equatorial 8s epimer.[16,18,51] Average upfield shifts of $\Delta\delta$ 4.1 (7), 6 (1), and 0.9 (4) ppm were found for piperidine-ring carbons β, γ, and δ to N-CH_3, respectively, in salts of **1–3**, **6** and **7**. The ethano bridge C(6,7) atoms γ to N-CH_3 in the axial 8r diastereomers are correspondingly shifted downfield by an average of $\Delta\delta$ 2.0 (6) ppm relative to those in the equatorial 8s epimers. As expected, axially oriented N-CH_3 are shifted upfield by an average of $\Delta\delta$ 7.3 (2) ppm versus their equatorially disposed counterparts. Scopolamine hydrobromide (**2**·HBr) is an exception, since the N-CH_3 shift difference is decidedly greater [$\Delta\delta$ 14 (1) ppm, D_2O or CD_2Cl_2 solution]. Equatorial N-CH_3 in **2e**·HBr is particularly deshielded in both solvents [46 (1) ppm] relative to more typical 40 (1) ppm values found for N-CH_3 in **1e**·HBr. Close proximity of the scopolamine equatorial N-CH_3 nucleus to the transannular oxirane oxygen atom [r(eq-NC···O) = 2.76 Å in scopolamine model compound **13a**][52] is likely to be behind this additional deshielding of $\Delta\delta$ ca. 5.75 ppm. Similar 33 (1) ppm values were noted for the more distant axial N-CH_3 in both scopolamine, atropine cations (see Table III).

Proton NMR Spectral Parameters. In CD_2Cl_2, *all* five tropane salts exhibited two NHCH_3 doublets in the same ratio (ca. 18:1)! Assignments of equatorial, axial descriptors to the two unequally populated ^1H NMR species were made via ^{13}C NMR correlation with the minor,major N-CH_3 8r,s diastereomers in CD_2Cl_2. Tropine skeleton axial,equatorial N-CH_3 ^1H chemical shift differences exhibit the converse relationship to those for ^{13}C N-CH_3, as expected. Using quaternization studies, Closs[15] demonstrated that the axial N-CH_3 in a series of tropine and pseudotropine derivatives was $\Delta\delta$ ca. 0.17 (5) ppm deshielded vis-â-vis the average value observed for the equatorial set. Similarly, in our ^1H NMR (CD_2Cl_2) SEL study, the axial N-CH_3 in **1a,e,3a,e**·HX was $\Delta\delta$ 0.13 (1) ppm deshielded vis-â-vis equatorial N-CH_3. However, it is the unequivocally characterized equatorial N-CH_3 in the **2a,e**-HBr pair that is now $\Delta\delta$ 0.05 ppm deshielded relative to its externally diastereotopic axial counterpart. The neighboring oxirane oxygen preferentially deshielded the closer equatorial N-CH_3 compared to the more distant axial N-CH_3 (model compound **13a** minimum nonbonded distance r(NCH···O) = 2.22 Å [eq-CH] vs 4.36 Å [ax.-CH]). The oxirane oxygen substituent effect can also be seen from the known ^1H NMR spectral parameters for methiodide quaternary ammonium salt[23,53] and amine N-oxide[49]

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(52) (8s)-*O*-Benzoyltropine hydrochloride (**12e**)[44] and (9s,C$_\alpha$-*S*)-*N*-butylhyoscinium bromide (**13a**)[48] are models for (8s)-atropine and (9r,C$_\alpha$-*S*)-scopolamine geometry, respectively, since hydrogen coordinates were not given for **1e**·HBr[39b,40] nor for **2a**·HBr.[39a]

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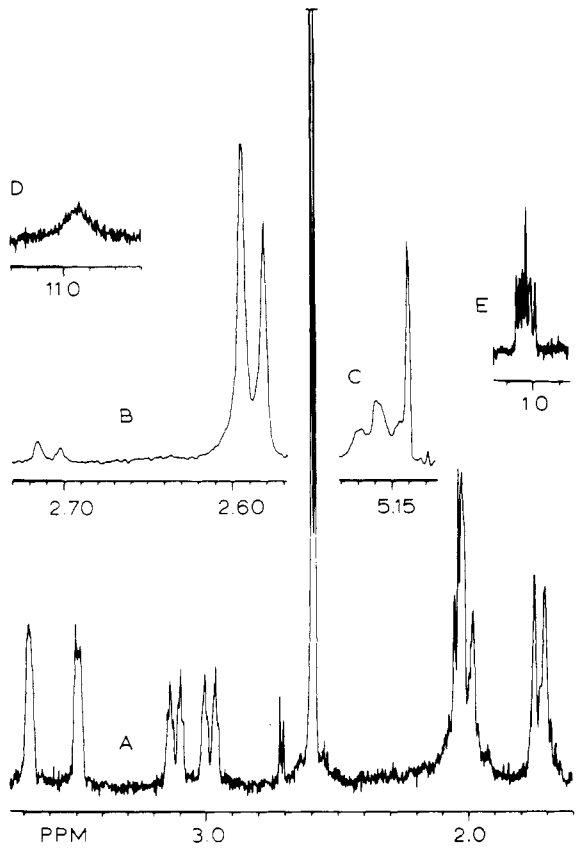

Figure 3. Trace A: 1.60–3.75 ppm ^{1}H NMR (400.1 MHz, 294 K) aliphatic region of (8*r*,*s*)-homatropine hydrobromide (**3a**,**e**·HBr) *N*-CH_3 diastereomeric mixture, **a**:**e** ratio ca. 1:18 (in CD_2Cl_2). Trace B: 2.57–2.73 ppm axial,equatorial *N*-CH_3 region (0.25× reduced intensity). Trace C: 5.12–5.18 ppm region showing H(3) overlapped with H(α), (0.25× reduced intensity). Trace D: N–*H*. Trace E: 0.85–1.15 ppm H(61) region.

scopolamine diastereomers. Equatorial *N*-CH_3 is $\Delta\delta$ 0.25 (4) ppm[54] deshielded by the cis-1,3-transannular oxirane oxygen, as seen by comparisons of scopine vs tropine derivatives: [**2e**·HBr vs **1e**,**3e**·HX; **2**·MeI vs **1**·MeI; **5**·MeI or 6-*exo*-hydroxytropine methiodide vs **1**·MeI; and **2**·(9*r*)-*N*-oxide vs **1**·(8*r*)-*N*-oxide]. Similar comparisons of the oxirane oxygen substituent effect on the farther axial *N*-CH_3 show smaller mixed shielding/deshielding of ±0.05 (4) ppm.[54] X-ray crystallographic studies[49,55] removed the earlier confusion[23] regarding oxirane substituent effects in scopine (**5**) type derivatives.

A similar ca. 18:1 equatorial:axial diastereomeric ratio in CD_2Cl_2 for all five (**1–3**, **6** and **7**) salts suggests that the 2-*exo*-carbomethoxy substituent in *l*-cocaine hydrochloride (**7**·HCl) does not present a very formidable cis-1,3 steric barrier to axial *N*-CH_3 in this solvent. The X-ray crystallographic molecular geometry for *l*-cocaine methiodide[56] (**14**) shows that torsion angle C(1)–C(2)–$C_{carbonyl}$–$O_{methoxy}$ opened up to −46° [putatively to accommodate the axial (*pro-R*)-CH_3] relative to the −17° value found for crystalline **7e**·HCl[42] (axial N*H*). The axial (*pro-R*)-CH_3 in **14** lies above the plane delineated by C=O and $O_{methoxy}$ [nonbonded distances r(NCH_3···OCH_3) = 3.31 Å, (NCH_3···C=O) = 2.98 Å, and r(NCH_3···O=C) = 3.35 Å]. The 1,3-cis-transannular C=O bond magnetic anisotropy shields **7a**·HCl axial *N*-CH_3 vs **7e**·HCl equatorial *N*-CH_3 (see Table I). Nonobservance of an axial **7a**·HCl 8*R* diastereomer in the ^{13}C NMR SEL spectrum recorded in D_2O suggests a different behavior for the axial CO_2CH_3 moiety in this protic, more polar medium as compared to that found in CD_2Cl_2.

Diastereotopicity for each of the H(61,71) and H(62,72) pairs and the efficient dispersion of shielding constants by the high magnetic fields employed in this study enabled the determination of hitherto unreported vicinal and geminal coupling constants between ethano bridge synperiplanar and anticlinal[57] nuclei (see Table II). ^{1}H coupling networks were identified by using COSY-30 2D NMR[58] together with selective 1D ^{1}H NMR decoupling. Nuclei in one diastereotopic half of the dissymmetrized tropane skeleton gave rise to the lower field signals in four out of five pairs of internally diastereotopic protons [H-(1,5), H(21,41), H(22,42), and H(62,72)], while those in the other half of the molecule gave rise to the higher field signals within each pair. For the purpose of this discussion, coupling networks within each of the tropane halves were differentiated by assigning the higher field signals in each pair as H(1), H(21), H(22), and H(72) (labeling experiments must be performed to ascertain their correct location in either the 1*R* or 5*S* half). COSY-30 showed that the lower field signal of the H(61,71) endo pair was linked to the higher field signal in the H(62,72) exo pair via a cross-peak pattern perpendicular to the diagonal. On the basis of the negative coupling constant, the lower field signal in the H(61,71) pair and the higher field signal in the H(62,72) pair for atropine mesylate (**1e**·CH_3SO_2OH) were respectively assigned as H(71) and H(62). The nonobservance of measurable ^3J(1–71) and ^3J(5–61) coupling constants for scopolamine hydrobromide (**2e**·HBr) is consistent with ±77 (3)° H(1)–C(1)–C(7)–H(71) [H(5)–C-(5)–C(6)–H(61)] torsion angles in the solid-state scopolamine model **13a**.

The conformation of the tropic acid ABX nuclei[59] for both atropine and scopolamine cations in D_2O has been determined based on averaged rotamer population J(AB) and J(AX) (or J(BX)) coupling constants.[19] With Dimitrov's method,[59] our SEL results [e.g., $^2J(\beta\beta')$ = −11.6, $^3J(\alpha\beta)$ = 9.4, and $^3J(\alpha\beta')$ (the J(AB)) = 5.2 Hz for **2e**·HBr] show the antiperiplanar-[(C_α)-phenyl,(C_β)-hydroxyl] conformation to be the major species (ca. 74%) in CD_2Cl_2 solution, as it is for the **2a**·HBr epimer in D_2O,[19] and in the crystalline[37] state. The **2e**·HBr antiperiplanar-[(C_α)-carboxyl,(C_β)-hydroxyl] and gauche–gauche conformations in CD_2Cl_2 were calculated to be ca. 21% and ca. 5%, respectively.

Hitherto unreported long-range ^4J(22–42) and ^4J(41–62) coupling constants (signs not determined) for cocaine and homatropine cations are consistent with extended "W"-type arrangements within piperidine-ring chair conformations for the ecgonine and tropine moieties in CD_2Cl_2 solution (see Table II). Carroll et al.[30] reported ^4J(22–42) = 0.8 Hz for free base **7**. Evidence for a chair conformation also comes from the similar 3–4 Hz ^3J(1–21),^3J(5–41) and ^3J(1–22),^3J(5–42) coupling constants expected for bridgehead protons mutually synclinal[57] (gauche) to adjacent C(2) or C(4) methylene protons. Ring deformation at C(3) in the cationic chair-type tropine and scopine moieties [presumably due to nonbonded interactions between the endo H(61,71) protons and the axial oxygen atom at C-(3)][19,22] is evident from lack of observed coupling between

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H(32) and either H(22) or H(42), while ^3J(21–32) and ^3J(32–41) values are 4–5 Hz. Single-crystal X-ray diffraction determined torsion angles in **12e** and **13a** provide models[52] for semiquantitative Karplus-type[60,61] comparisons with the chair conformation piperidine-ring geometries of atropine and scopolamine cations in solution. Coupling constants ^3J(21–32),^3J(32–41) and ^3J(22–32),^3J(32–42) noted for **1e** and **2e** salts are in accord with relevant torsion angles for crystalline **12e** and **13a**: H(21)–C(2)–C(3)–H(32) [H(32)–C(3)–C(4)–H(41)] = ±36 (3)° and H(22)–C(2)–C(3)–H(32) [H(32)–C(3)–C(4)–H(42)] = ±80.1 (1)°.[44,48] Some distortion at C(3) is also noted for the ecgonine ring of cocaine hydrochloride (**7e**·HCl) in CD_2Cl_2. Evidence for this is provided by the synclinal (gauche) ^3J(22–31),^3J(31–42) coupling constants, whose magnitudes are consistent with ±41.4 (4)° H(22)–C(2)–C(3)–H(31) [H(31)–C(3)–C(4)–H(42)] torsion angles in crystalline **7e**·HCl.[42]

Stability of Equatorial and Axial *N*-Methyl Scopolamine 9*r*,*s* Diastereomers. The *N*-CH_3 8*r*,*s* diastereomeric equilibrium for atropine sulfate/mesylate (**1**·$0.5H_2SO_4$/**1**·CH_3SO_2OH), homatropine hydrobromide (**3**·HBr), and benztropine mesylate (**6**·HCH_3SO_2OH)] at SEL ^1H and ^{13}C NMR conditions was overwhelmingly in favor of the equatorial *N*-CH_3 8*s* isomer both in D_2O (**e**:**a** ratio ca. 7:1) and in CD_2Cl_2 (**e**:**a** ratio ca. 18:1). Cocaine hydrochloride (**7**·HCl) also showed preponderance of the equatorial 8*s* diastereomer [<95% **e** (D_2O); **e**:**a** ratio ca. 18:1 (CD_2Cl_2)]. In addition, ^{13}C NMR spectra ($CDCl_3$) of the free bases clearly showed an overabundance of the equatorial *N*-CH_3 diastereomer [based on FEL δ-values characteristic for equatorial *N*-CH_3: 40.0 (4) ppm[20,28,29,31] (**1**) and 41.3 (3) ppm[28,29,30,31] (**7**)].

(9*r*)-Scopolamine hydrobromide hemihydrate (**2a**·HBr) has been the odd man out in terms of its solid-state axial *N*-CH_3 9*r* orientation.[39a] The above-mentioned (9*s*)-*N*-butyl quaternary ammonium salt **13a** and **2**·(9*s*)-*N*-oxide also have axial *N*-CH_3, but both were the *kinetic* products of preferential equatorial attack.[53] In three ^{13}C NMR studies on the scopolamine cation in D_2O, only one (Avdovitch and Neville[31]) correctly assigned the *N*-CH_3 absorbance (34.31 ppm) both in terms of its chemical shift and its axial 9*r* stereochemical disposition. These authors did not note the observance of the equatorial *N*-CH_3 9*s* diastereomer.[31] The same authors assigned *axial* *N*-CH_3 stereochemistry to scopolamine free base despite its ^{13}C NMR FEL chemical shift of 42.45 ppm[31] in $CDCl_3$. Similar FEL-averaged ^{13}C NMR chemical shifts were reported prior to their study [43 (1) ppm[20,29]]. Comparison of these FEL results with our ^{13}C NMR SEL findings for the cation clearly points to equatorial *N*-CH_3 9*s* stereochemistry for the major free-base equilibrium species. ^1H NMR FEL spectral results also support this assignment for the scopolamine free base major isomer. The *N*-CH_3 FEL signal in scopolamine (**2**) is Δδ 0.26 (1) ppm deshielded relative to the corresponding protons in atropine (**1**) [in either DMSO-d_6[23] or $CDCl_3$[32]].

Surprisingly, our ^1H and ^{13}C NMR studies (CD_2Cl_2) at the SEL for *N*-CH_3 epimerization showed conversion of the *axial* *N*-CH_3 species in crystalline scopolamine hydrobromide[39a] (**2a**·HBr) into the same ca. 18:1 **e**:**a** diastereomeric mixture as noted for atropine, homatropine, benztropine, and cocaine salts. In ^{13}C NMR SEL spectra (D_2O), as opposed to previous reports, equatorial *N*-CH_3 (9*s*)-scopolamine hydrobromide (**2e**·HBr) was now observed along with the **2a**·HBr epimer. Equilibrium populations were *reversed* in this medium (ca. 1:18 **e**:**a** ratio).

The molecular geometry of axial *N*-CH_3 crystalline (9*s*,C_α)-*N*-butylhyoscinium bromide methanol solvate[48] model compound **13a** does *not* show less severe piperidine-ring nonbonded cis-1,3-diaxial interactions vis-à-vis those found in the pyrrolidine cycle. Minimum nonbonded distances calculated for **13a** are *r*(ax-NC*H*···H(21) [H(41)] = 2.09 [2.18] Å while *r*(eqNC*H*···O) = 2.22 Å. The oxirane oxygen is not expected to present an overly large steric barrier to an equatorial *N*-CH_3.

Eliel and co-workers recently made an extensive study of *N*-CH_3 diastereomerization in monocyclic *N*-CH_3 piperidine derivatives.[63] Moreover, opiate agonists and antagonists have also been found in acidic aqueous medium (or CD_2Cl_2) to exist in two diastereomeric forms differing in their *N*-alkyl orientation.[64–67] Invocation of the Curtin–Hammett principle[68] reminds us that the disparate *N*-CH_3 isomer ratio provides no clues as to the receptor binding preference. Recently, using intramolecular transferred NOE measurements, Glasel and Borer showed that opiate agonists and antagonists had different *N*-alkyl configurations at the binding site of a specific anti-opiate monoclonal antibody fragment.[69] The axial diastereomer of the opiate antagonist nalorphine was found as the bound form, while the equatorial epimer was the preponderant solution species (ca. 5:1).[69]

Additional studies on *N*-CH_3 epimerization in pharmacologically active agents are currently in progress.

Experimental Section

Atropine sulfate, scopolamine hydrobromide, homatropine hydrobromide, and benztropine mesylate were purchased from Aldrich Chemical Co. Atropine sulfate U.S.P. XIX and scopolamine hydrobromide U.S.P. XIX were gifts of Plantex (Israel) Ltd. *l*-Cocaine hydrochloride was obtained from Sigma Chemical Co. Atropine free base was obtained as a gift from Merck Frosst Canada Inc. Atropine mesylate was prepared by addition of a stoichiometric quantity of methanesulfonic acid to a solution of atropine free base in 1:1 ether/dichloromethane.

^1H and ^{13}C NMR spectra (9.4 T, CD_2Cl_2, sealed 5- and 10-mm sample tubes, respectively) were obtained at 400.1 and 100.6 MHz, respectively, on a Bruker WH-400 Fourier transform spectrometer. ^1H NMR spectra (CD_2Cl_2) were also recorded at 200.1 and 299.9 MHz on Varian XL-200 and XL-300 Fourier transform spectrometers (4.7 and 7.0 T, respectively). Solid-state ^{13}C NMR (50.3 MHz) were recorded on the Varian XL-200 spectrometer operating in the CP-MAS mode using the TOSS (total suppression of spinning sidebands)[70] technique. Hexamethylbenzene (132.1 ppm) was used as an external secondary reference for the solid-state spectra. Evolution delay periods of 24 μs were used in solid-state dipolar dephasing experiments on atropine sulfate and scopolamine hydrobromide. ^{13}C NMR spectra (4.7 T, D_2O) were obtained at 50.3 MHz on a Bruker WP-200-SY Fourier transform spectrometer. The broad-band proton-decoupling technique was utilized for the ^{13}C NMR spectra, and the deuteriated solvent was

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used as an internal lock. Residual protio CH_2Cl_2 solvent absorbances were used as internal secondary references (relative to tetramethylsilane). The internal reference in D_2O solutions was 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt.

Diastereomeric ratio determinations in CD_2Cl_2 solutions were performed by integration of the externally diastereotopic ^1H NMR N-CH_3 signals. An indication of the diastereomeric ratio in D_2O solutions was obtained by integration of the externally diastereotopic ^{13}C NMR N-CH_3 signals.

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An Unusual Stereochemical Outcome of a Peroxyacid Epoxidation Reaction: Stereospecific Synthesis of (4′*R*)-Spiro[oxirane-2,4′-5′α-cholestan-3′β-ol]

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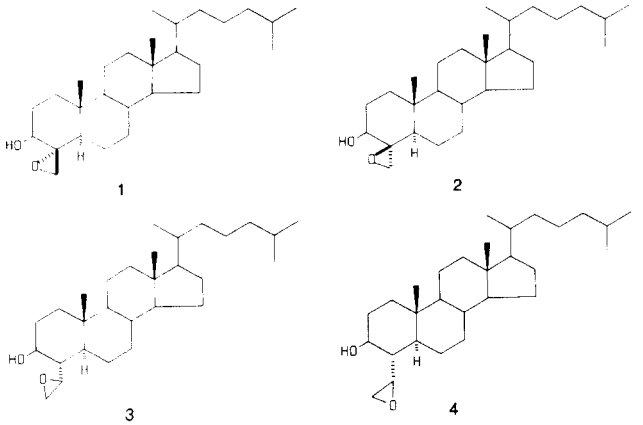
The epoxidation of 4-methylene-5α-cholestan-3β-ol (**10**) with *m*-chloroperoxybenzoic acid or with (+)- or (−)-diethyl tartrate/*tert*-butyl hydroperoxide–titanium tetraisopropoxide leads stereospecifically to (4′*R*)-spiro[oxirane-2,4′-5′α-cholestan-3′β-ol] (**1**). The relative stereochemistry of **1** has been established by X-ray crystallography. The lack of directional effect by the hydroxyl group in the epoxidation of the allylic alcohol **10** is unusual.

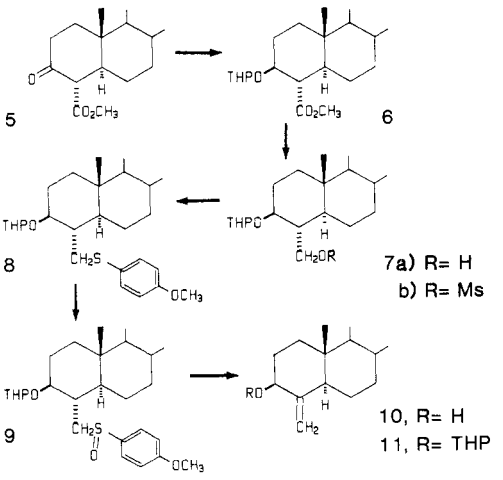
As part of a program aimed at the development of inhibitors of the cholesterol biosynthetic enzyme 4-methyl sterol oxidase[1] we required (4′*R*)- and (4′*S*)-spiro[oxirane-2,4′-5′α-cholestan-3′β-ol (**1** and **2**). We have shown[2]



previously that the homologous 4′α-(2(*S*)-oxiranyl)- and 4′α-(2(*R*)-oxiranyl)-3′β-hydroxy-5′α-cholestanes (**3** and **4**) are potent inhibitors of this enzyme system. This report describes the stereospecific synthesis of (4′*R*)-spiro[oxirane-2,4′-5′α-cholestan-3′β-ol] (**1**) and the assignment of its relative stereochemistry by X-ray crystallography.

The allylic alcohol **10** (Chart I) was chosen as the most convenient precursor of the spiro epoxides **1** and **2**. Alternative precursors such as 4-methylene-5α-cholestan-3-one[3] or 4-oxo-5α-cholestan-3β-ol are labile or difficult to homologate. It was intended to generate the epoxides **1** and **2** from the allylic alcohol **10** by appropriate asymmetric epoxidation procedures.[4] There is ample precedent for hydroxy-assisted organic peroxyacid epoxidation of allylic alcohols to furnish epoxides in which the stereochemistry of the epoxide is determined by the configuration of the hydroxy group.[5] Peroxyacid oxidation of 4-methylene-5α-cholestan-3β-ol (**10**) might therefore be expected to furnish predominantly the (4′*S*)-spiro epoxide

Chart I



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‡ National Institutes of Health.

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