cell lysates are diluted directly, and the virus yield is assayed by plaque formation on Vero cells. The number of plaque-forming units (PFU) of virus in the drug-treated cultures relative to that found in the drug-free condition is determined.

The *cytotoxicity* of various test compounds on the uninfected host Vero cells is determined. Vero cells in Dulbecco's medium (2.5 mL) supplemented with 10% fetal calf serum are added to eight 25-cm^2 Falcon flasks at a concentration equivalent to 0.1 confluency for each compound under assay. After incubation at 37 °C in 5% CO_2–95% air for 1 day, the test compound, dissolved in 2.5 mL of the above growth medium, is added, and two flasks are harvested immediately by decanting the medium, washing once with 5 mL of buffered saline, and then incubating at 37 °C for 15 min with a 5 mL solution of trypsin (0.125%) and EDTA (0.02%). The cells dislodged from the flask by this latter procedure are generally in clumps and are dispersed by repeated forceful pipetting of the suspension. Trypan blue solution (0.2 mL) is added, and the number of cells are counted with a haemocytometer. Each day for the next 3 days, two of the remaining flasks are harvested in the manner just described for determination of cell number.

Acknowledgment. This research was supported by USPHS Grants CA-45410 and CA-28852. We also acknowledge the support of Northeast NMR Facility at Yale University for the high-resolution NMR spectra, made possible by a grant from the Chemical Division of the NSF (Grant CHE-7916210).

Registry No. 1, 62138-01-6; 2, 62102-29-8; 3, 59967-83-8; 4, 69975-22-0; 5, 111795-56-3; 6, 111795-57-4; 7, 111795-58-5; 8, 111795-59-6; 9, 111795-60-9; EtOCH=CHCONCO, 57796-78-8.

2-Methyl-1,3-dioxaazaspiro[4.5]decanes as Novel Muscarinic Cholinergic Agonists

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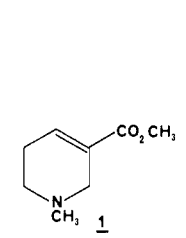
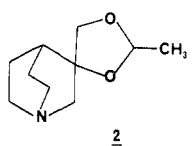
Many nonquaternary ammonium muscarinic agonists have been developed over the last few years, but most of the existing compounds (e.g., arecoline, RS-86, AF-30) behave as weak partial agonists at cholinergic receptors in tissues of limited receptor reserve. The current paper describes the synthesis and biochemical assessment of analogues of AF-30 designed to have sufficient conformational freedom to allow greater receptor flexibility and hence activation. The new compounds and important standards were tested in a new biochemical assay designed to measure both receptor affinity and intrinsic activity of each compound and for their ability to stimulate phosphatidylinositol turnover in rat cerebral cortex. Two azaspirodecanes (**5a** and **5b**) were shown to have far greater predicted efficacy than AF-30.

The tertiary amines arecoline (**1**), AF-30 (**2**), and RS-86 (**3**) have been described as muscarinic agonists, but they behave as weak partial agonists incapable of eliciting a full response in some tissues.[1] For example, unlike the full agonists carbachol and acetylcholine, these compounds are capable of only marginally stimulating the breakdown of phosphatidylinositol (PI) in rat cerebral cortex, in vitro.[2]



1



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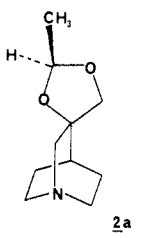
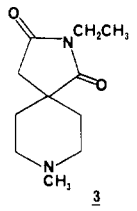
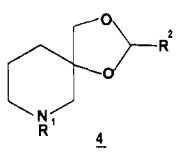


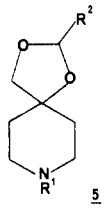
2a



3



4



5

4(a) $R^1=R^2=CH_3$; isomer A
(b) $R^1=R^2=CH_3$; isomer B
(c) $R^1=H; R^2=CH_3$; isomer A
(d) $R^1=H; R^2=CH_3$; isomer B

5(a) $R^1=R^2=CH_3$
(b) $R^1=H; R^2=CH_3$
(c) $R^1=CH_3; R^2=C_2H_5$

In attempting to explain this, it is necessary to consider those phenomena that describe the interaction of a ligand with its receptor.[3] The stimulation produced by an agonist in a given tissue is governed not only by the affinity of the compound for a specific receptor but also by its intrinsic activity (efficacy). This latter property depends on the number of receptors in that tissue and hence the efficiency of the coupling mechanism between receptor activation and the appropriate secondary messenger system. RS-86 has similar affinity for muscarinic receptors located in the heart and cortical tissues.[4] It produces a potent dose-dependent decrease in adenylate cyclase activity in cardiac membranes with a maximum response equivalent to that invoked by carbachol and other classical agonists having a quaternary ammonium group. In contrast, the compound produces a stimulation of phospholipid metabolism in slices of cerebral cortex that is less than 10% that of the maximum achieved by carbachol.[2] Thus, in tissues such as the cerebral cortex where there is relatively inefficient coupling between receptor activation and secondary messenger metabolism ("low receptor reserve"), the low efficacy of RS-86 is insufficient to produce a full agonist response.

Previous studies[5] in these laboratories have focussed on the completely rigid muscarinic agonist **2** and have shown that the most active stereoisomer (**2a**) has the 3*R*,2′*S* configuration indicated. By use of a recently developed[6]

‡ Chemistry Department.
† Biochemistry Department.

(1) Palacios, J. M.; Bolliger, G.; Closse, A.; Enz, A.; Gmelin, G.; Manalowski, J. *Eur. J. Pharmacol.* **1986**, *125*, 45.
(2) Freedman, S. B. *Br. J. Pharmacol.* **1986**, *87*, 29P.
(3) Kenakin, T. P. *Pharmacol. Rev.* **1984**, *36*, 165.
(4) Freedman, S. B.; Harley, E.; Iversen, L. L., manuscript in preparation.
(5) Saunders, J.; Showell, G. A.; Baker, R.; Freedman, S. B.; Hill, D.; McKnight, A.; Newberry, N.; Salamone, J. D.; Hirshfield, J.; Springer, J. P. *J. Med. Chem.* **1987**, *30*, 969.

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Table I. In Vitro Binding Data for the Azaspirodecanes 4 and 5 Compared with Standard Muscarinic Ligands

compound	n^a	NMS binding assay,[b] K_{app}[c] (range[d])	oxo-M binding assay,[f] K_{app}[c] (range[d])	ratio[g] (range[d])
carbachol	4	24 (18–26)	0.0058 (0.0022–0.0072)	4100 (3200–5400)
atropine	4	0.001 (0.00092–0.0012)	0.00048 (0.00040–0.00064)	2.1 (1.6–3.0)
arecoline (1)	3	6.2 (5.1–9.0)	0.011 (0.0098–0.012)	560 (520–750)
AF-30 isomer (2a)	4	4.9 (4.0–6.0)	0.035 (0.033–0.04)	140 (120–155)
RS-86 (3)	3	5.0 (4.3–5.4)	0.040 (0.033–0.046)	130 (100–160)
4a	4	>100,[e] 24% (13–34%)	>10,[e] 4% (3–5%)	
4b	4	>100,[e] 19% (12–23%)	>10,[e] 10% (7–12%)	
4c	4	>100,[e] 29% (24–35%)	>10,[e] 25% (21–29%)	
4d	4	>100,[e] 20% (17–25%)	>10,[e] 13% (8–17%)	
5a	6	25 (18–40)	0.032 (0.024–0.050)	780 (540–930)
5b	4	43 (36–56)	0.092 (0.072–0.12)	470 (360–600)
5c	6	5.3 (2.8–9.7)	0.22 (0.17–0.29)	24 (12–57)

[a] Each value in subsequent columns is the geometric mean of the number of independent experiments, n. Each experiment was performed in triplicate with dose–response curves measured at a minimum of four concentrations. [b] Displacement of [^{3}H]-*N*-methylscopolamine in rat cortex. [c] The apparent affinity constant corrected for radioligand occupancy and expressed as micromolar concentrations. [d] Since SEM values are inappropriate for geometrically meaned values, the range is provided as an indication of variability. [e] These compounds were tested at the concentration (μM) indicated with the percent inhibition of binding shown. [f] Displacement of [^{3}H]oxotremorine M in rat cortex. [g] The ratio of NMS/oxo-M K_{app}'s.

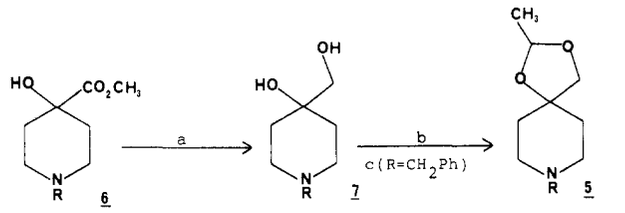
in vitro binding assay capable of measuring both affinity of a ligand for cortical muscarinic receptors and also predicting intrinsic activity of that ligand, **2** was shown to have comparable efficacy to that of RS-86. Although the rigidity of AF-30 may well contribute to the selectivity observed in one of its stereoisomers, this property may also limit its efficacy as an agonist since subtle but complimentary conformation changes in both receptor and ligand may be necessary to trigger the secondary messenger signal.

Both arecoline and RS-86 have been evaluated in the clinic[7] for their potential therapeutic benefit in the treatment of Alzheimer's disease and senile dementia of the Alzheimer type. Only arecoline showed a statistically significant cognitive improvement in Alzheimer's patients, but its extremely short duration of action renders its clinical use impractical.[8] This short half-life may be attributable to the rapid in vivo hydrolysis of the ester functionality. A compound having efficacy similar to or greater than that of arecoline but which is devoid of the metabolically labile ester moiety may therefore represent a superior clinical candidate. Although compounds with such a profile have been reported[9] in the oxotremorine series, nothing has been published regarding their potential clinical utility. The purpose of this study was to prepare close analogues of **2** such as **4** and **5** with sufficient conformational freedom to allow greater receptor flexibility and hence activation.[10]

Synthetic Chemistry

8-Methyl-1,3-dioxa-8-azaspiro[4.5]decanes (**5a** and **5c**) were prepared from the known hydroxy ester[11] **6a** by the route summarized in Scheme I. The method of choice for formation of the dioxolane ring from the diol **7a** involved acid-catalyzed transketalization of the appropriate aldehyde diethyl acetal. Indeed, for enolizable aldehydes other than acetaldehyde, the published method[12] using BF_3·Et_2O and the aldehyde itself yielded only a polymeric product. Presumably, higher aldehydes in which the enol form is stabilized by σ donation from the alkyl substituent preferentially undergo aldol polymerization under these conditions. The 8-desmethyl analogue **5b** was prepared by essentially identical chemistry with the hydroxy ester **6b** as starting material to give **5d**, deprotection of which was achieved by hydrogenolysis over palladium hydroxide on carbon.

Scheme I[a]



[a] Reagents: (a) $LiAlH_4$, THF; (b) $CH_3CH(OEt)_2$, TsOH; (c) 20% $Pd(OH)_2$/C, EtOH, H_2.

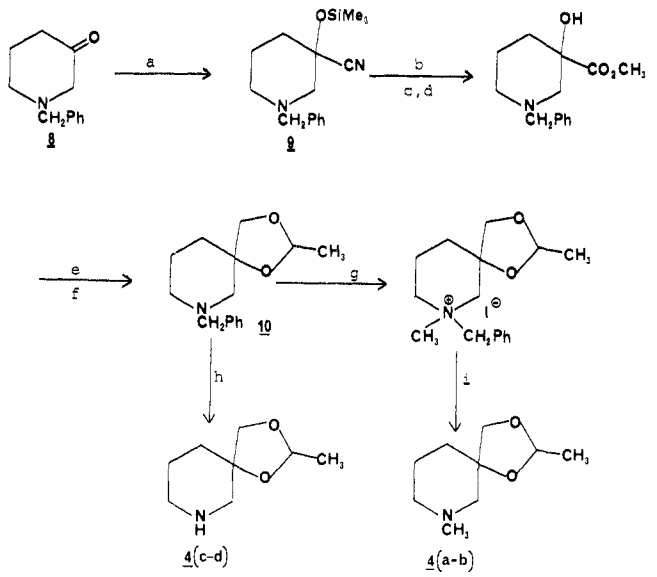
The two diastereomeric 2,7-dimethyl-1,3-dioxa-7-azaspiro[4.5]decanes (**4a,b**) and their 7-desmethyl analogues (**4c,d**) were prepared from 1-benzyl-3-piperidone[13] (Scheme II) via the intermediacy of the *o*-(trimethylsilyl)cyanohydrin **9**. Attempted cyanohydrin formation under aqueous conditions was unsuccessful because the 3-piperidone salt (**8**) exists exclusively as a stable hydrate, which fails to react as a typical ketone. Elaboration of **9** following the usual procedure gave the intermediate spirodioxolane **10**, which afforded the target compounds as illustrated (Scheme II). Although the diastereomers (**4a,b**) and also (**4c,d**) could be readily separated by chromatography on alumina, the relative stereochemistry of each could not be determined by proton NOE NMR experiments.

Results and Discussion

Each compound was assessed in an in vitro binding assay[6] designed to measure affinity and predict efficacy

(6) Freedman, S. B.; Harley, E.; Iversen, L. L. *Br. J. Pharmacol.* **1987**, *90*, 80P.
(7) For a recent review, see: Hollander, E.; Mohs, R. C.; Davis, K. L. *Br. Med. Bull.* **1986**, *42*, 97.
(8) Christie, J.; Schering, A.; Ferguson, J. *Br. J. Psychiatry* **1981**, *138*, 46.
(9) Fisher, S. K.; Figueiredo, J. D.; Bartus, R. T. *J. Neurochem.* **1984**, *43*, 1171.
(10) After this work had been completed, publication of these compounds as the subject of a patent application (EP 189370) became known. However, the compounds were prepared by different synthetic methodology and no biochemical data or structure–activity discussion was presented.
(11) Grob, C. A.; Renk, E. *Helv. Chim. Acta* **1954**, *37*, 1689.
(12) Cohen, S.; Fisher, A. U.S. Patent 4 104 397, 1978.
(13) Krogsgaard-Larsen, P.; Hjeds, H. *Acta Chim. Scand., Ser. B* **1976**, *B30*, 884.

Scheme II[a]



[a] Reagents: (a) TMSCN, ZnI_2; (b) concentrated HCl; (c) MeOH–HCl (gas); (d) Na_2CO_3; (e) $LiAlH_4$; (f) $CH_3CH(OEt)_2$, TsOH; (g) CH_3I, $(CH_3)_2CO$; (h) 20% $Pd(OH)_2$ on carbon, EtOH, H_2; (i) 10% Pd/C, EtOH, H_2.

at cortical muscarinic receptors (Table I). By use of [^{3}H]oxotremorine M (oxo-M) to label the high affinity state of the cerebral cortex muscarinic receptors and [^{3}H]-*N*-methylscopolamine (NMS) to label predominantly the low state, it was possible to measure affinity of each compound for either state of the receptor. Since agonists recognize preferentially the high affinity state displaying much lower affinity in the NMS assay, whereas antagonists show similar affinity in both assays, the ratio of the affinities of a given compound (NMS/oxo-M ratio) gives a measure of its cortical intrinsic activity. The classical agonist carbachol, having a quaternary ammonium group, showed much higher affinity in the oxo-M assay relative to the NMS assay giving a high NMS/oxo-M ratio typical of an agonist with high intrinsic activity. Conversely, atropine displayed a characteristic antagonist profile having approximately equal affinity for both states of the muscarinic receptor and, therefore, a NMS/oxo-M ratio close to unity.

Comparison of such binding data obtained with the azaspirodecanes of this study with **2a** reveals that two compounds (**5a,b**) have a significantly greater NMS/oxo-M ratio than **2a**, suggestive of a greater level of intrinsic activity for these molecules at cortical muscarinic sites. In both cases, the increased ratio is predominantly the result of diminished affinity at the NMS site rather than an increase in affinity for the state labeled by oxo-M. On the other hand, the ethyl analogue **5c** showed a marked increase in affinity for the NMS-labeled site with a concomitant decrease in binding at the oxo-M site, thereby converting its behavior into that typified by compounds of low intrinsic activity such as antagonists. This remarkable change in binding characteristics is entirely consistent with other series of muscarinic agonists[14] and indicates the importance of lipophilic binding in the region occupied by the ethyl (or larger) group. Although still markedly inferior to carbachol (Table I), **5a** represents a significant improvement over agonists such as RS-86 and AF-30 in terms of predicted efficacy at cortical muscarinic receptors.

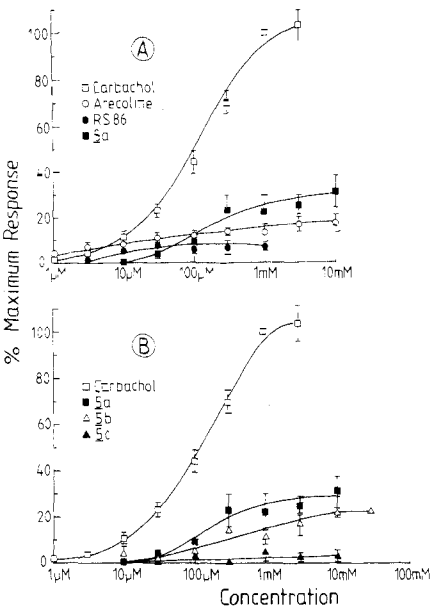


Figure 1. Effect of muscarinic ligands on phosphatidylinositol turnover in rat cerebral cortical slices. Results are expressed as the percentage of maximum response produced by 1 mM carbachol. Each point is the mean and the standard error of the mean of between three and six independent experiments.

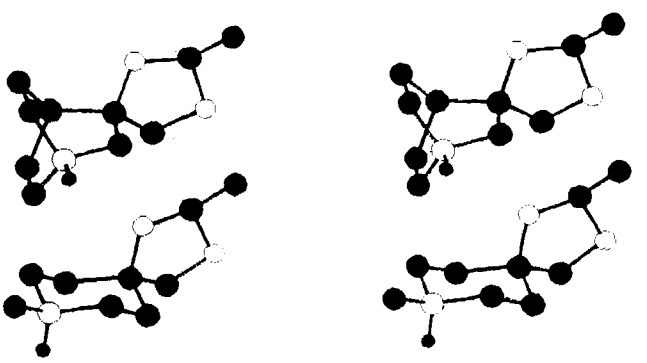


Figure 2. Stereoscopic view of **2a** and **5ai** in the same relative orientation.

The ability of muscarinic agonists to stimulate the breakdown of the inositol phospholipids has been used previously to assess the efficacy of muscarinic agonists in guinea pig cerebral cortex.[15] Because of the very limited number of spare receptors in this tissue, only compounds with high intrinsic activity are able to produce more than a minimal response; partial agonists are, therefore, readily identified. Carbachol (NMS/oxo-M ratio 4100) produced a dose-dependent stimulation of inositol monophosphate accumulation (see Figure 1A) with an EC_{50} of 140 μM. In contrast, RS-86 and the single AF-30 isomer (**2a**, data not shown), which had much lower NMS/oxo-M ratios (130), had only limited efficacy, producing only 8% and 10%, respectively, of the maximal response seen for carbachol. Arecoline was significantly better with a maximal response of 16%.

The ability of **5a–c** to stimulate cortical phosphatidylinositol turnover is shown in Figure 1B. Compound **5c** had very poor efficacy, producing only a 5% response whereas **5a** and **5b**, with significantly higher NMS/oxo-M ratios, produced maximal responses of 30% and 23% relative to carbachol. These latter compounds are therefore significantly more efficacious than RS-86 and AF-30, and **5a**

(14) Sauerberg, P.; Larsen, J.-J.; Falch, E.; Krogsgaard-Larsen, P. *J. Med. Chem.* **1986**, *29*, 1004.

(15) (a) Fisher, S. K.; Bartus, R. T. *J. Neurochem.* **1985**, *45*, 1085. (b) Freedman, S. B. *Br. J. Pharmacol.* **1986**, *87*, 29P.

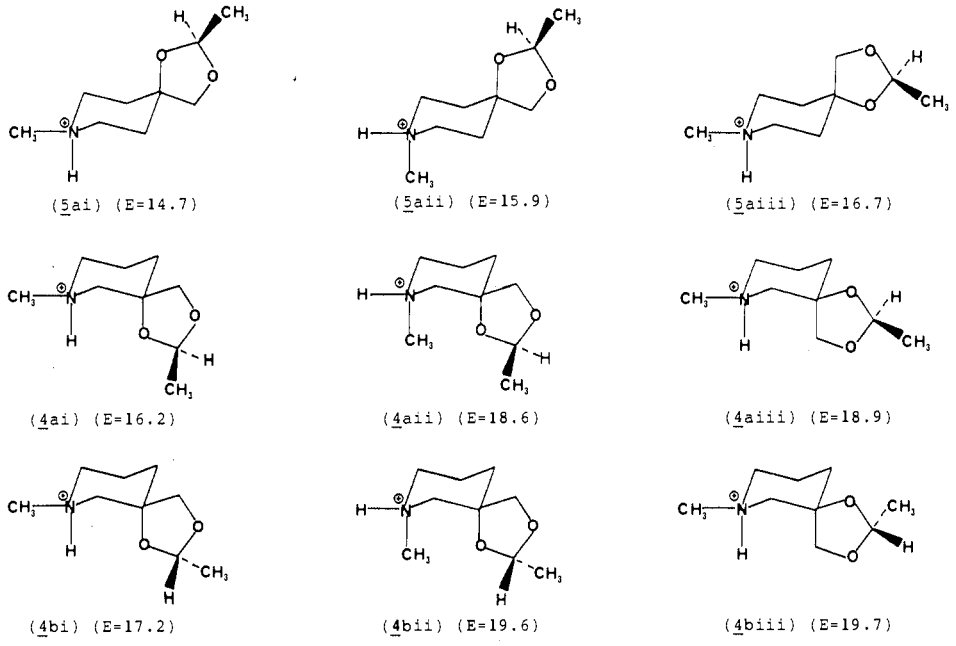


Figure 3. Lowest energy forms of 4a, 4b, and 5a from molecular mechanics calculations. All conformations of each isomer were considered in the analysis, but those not displayed had significantly higher energy. The relative stereochemistry of 4a and 4b has been arbitrarily assigned. No comparison of absolute energies between 4a, 4b, and 5a is intended. Figures in brackets are calculated energies in kcal Mol^{-1}.

compares favorably with arecoline in terms of receptor efficacy although it has 3- to 4-fold lower affinity.

Since at first sight the 7-azaspirodecanes (**4**) appeared to be more closely related to **2** than the 8-aza series (**5**), a rationalization of the inactivity of these compounds was sought. In order to compare **4a**, **4b**, and **5a** with the most active stereoisomer of AF-30 (**2a**, see Figure 2), it was first necessary to ascertain all low-energy conformers for **5a** and for both diastereoisomers **4a** and **4b**. Since it is the protonated form of these tertiary amines that is recognized by the muscarinic receptor, all calculations were performed on the protonated species. By use of a molecular mechanics-based procedure,[16] energies were computed for all reasonable ring conformations of each diastereomer, and the most energetically favored are depicted in Figure 3. In each case, the minimum-energy form was characterized by an equatorial NCH_3 group, a chair piperidine ring, and an equatorial CH_2/axial O arrangement at the spiro junction. Molecular superimpositions were attempted[17] between **2a** and all conformations of each enantiomer of **4a**, **4b**, and **5a** within 2 kcal mol^{-1} of the respective global minima. Both low-energy forms of **5a** (**5ai** and **5aii**) gave adequate fits with **2a** (exemplified in Figure 2) wherein the electrostatic interaction between N^+–H of **2a** and the receptor[18] would be unimpeded by the steric bulk of the piperidine ring of **5a**. Although a good overlay can be achieved between **2a** and a conformer of **4a** with an axial CH_2, this form (**4aiii**) is energetically disfavored with respect to the global minimum energy form, and this state will not be significantly populated at ambient temperature. Since no satisfactory superimposition could be achieved between energetically favored forms of **4a** and **4b** with **2a**, this would account for the lack of activity in these compounds.

In summary, therefore, **5a** represents an important advance in the design of new muscarinic agonists having greater intrinsic activity than many other known ligands devoid of a quaternary ammonium entity and is at least as efficacious as arecoline.

Experimental Section

Chemical Methods. General Directions. Except where otherwise stated, the following procedures were adopted: all ^1H NMR spectra were recorded at 360 MHz on a Bruker AM360 instrument, mass spectra with a VG 70-250 mass spectrometer, and infrared spectra on a Perkin-Elmer 782 IR spectrometer. GC was performed on a 12-m SE30 capillary column with a Perkin-Elmer gas chromatograph (8320). Organic solvents were purified when necessary by the methods described by D. D. Perrin, W. L. F. Armarego, and D. R. Perrin (*Purification of Laboratory Chemicals*; Pergamon: Oxford, 1966). Petroleum ether (PE) refers to that fraction having a boiling point range of 60–80 °C. All solutions were dried over potassium carbonate and evaporated on a Büchi rotary evaporator at reduced pressure. Thin-layer chromatography and preparative chromatography were carried out using plates (Merck Art. 5550) and gravity columns (Merck Art. 1077, activity Brockman Grade III), respectively. Melting points are uncorrected.

4-Hydroxy-4-(hydroxymethyl)-1-methylpiperidine (7a). A solution of 4-hydroxy-4-(methoxycarbonyl)-1-methylpiperidine (**6**)[11] (55.68 g, 0.32 mol) in dry THF (350 mL) was added over 1 h to a suspension of $LiAlH_4$ (17.0 g, 0.45 mol) in dry THF (200 mL) stirred under nitrogen. After 4.5 h at reflux, the mixture was cooled, and EtOAc (50 mL) was added over 15 min followed in turn by H_2O (17 mL), 2 N NaOH (17 mL), and finally further H_2O (51 mL). The mixture was filtered through Hyflo, and the filtrate was evaporated to give **7a** as a pale yellow gum (34.2 g, 74%). The hydrochloride salt: mp 137–139 °C; R_f 0.10 in CH_2Cl_2/MeOH (5:1) on alumina plates. Anal. ($C_7H_{15}NO_2 \cdot HCl \cdot 0.2H_2O$) C, H, N.

2,8-Dimethyl-1,3-dioxa-8-azaspiro[4.5]decane Hydrochloride (5a). *p*-Toluenesulfonic acid monohydrate (28.0 g, 0.147 mol) was heated under reflux in toluene (140 mL) with a Dean and Stark trap. After the majority of the toluene had been removed by distillation, the anhydrous acid was cooled to room temperature. To this was added a solution of the foregoing diol (18.0 g, 0.124 mol) in dry 1,4-dioxane (70 mL) followed by acetaldehyde diethyl acetal (74 mL, 0.52 mol), and the reaction mixture was heated under reflux for 8 h. The residue obtained

(16) Using the OPTIMOL procedure (based on a molecular mechanics force field) within the Merck Molecular Modelling facility written by Dr. T. Halgren, Rahway (unpublished).
(17) Using the CHEMX program, Chemical Design Ltd., Oxford, U.K.
(18) Schulman, J. M.; Sabio, M. L.; Disch, R. L. *J. Med. Chem.* 1983, *26*, 817.

after evaporation of the solvents was partitioned between CH_2Cl_2 (250 mL) and H_2O (350 mL) containing Na_2CO_3 (30 g). The organic layer was separated, and the aqueous layer was reextracted with CH_2Cl_2 (100 mL). The combined organic extracts were washed with H_2O (100 mL), dried, and then evaporated to give an orange gum (19.5 g). Purification by column chromatography on neutral alumina yielded the free base as a yellow oil (8.92 g, 42%). The hydrochloride salt: mp 111–113 °C dec; R_f 0.60 in CH_2Cl_2/MeOH (9:1) on alumina plates; MS, m/z 171 (M^+, free base); ^1H NMR (D_2O) δ 1.41 (3 H, d, J = 5 Hz, $OCHCH_3$), 1.8–2.15 (4 H, m, 6-CH_2 and 10-CH_2), 2.90 (3 H, s, NCH_3), 3.25 and 3.52 (each 2 H, each m, 7-CH_2 and 9-CH_2), 3.77 and 3.91 (each 1 H, each d, J = 9 Hz, OCH_2), and 5.23 (1 H, q, J = 5 Hz, $OCHCH_3$). Anal. ($C_9H_{17}NO_2$·HCl·0.3H_2O) C, H, N.

4-Hydroxy-4-(hydroxymethyl)-1-(phenylmethyl)piperidine (7b). A 43.50-g (82%) sample of 7b was obtained from 4-hydroxy-4-(methoxycarbonyl)-1-(phenylmethyl)piperidine[12] (60 g, 0.24 mol) and $LiAlH_4$ (10 g, 0.26 mol) as described for 7a. The hydrochloride salt: mp 60–65 °C; R_f 0.30 in CH_2Cl_2/MeOH (5:1) on alumina plates. Anal. ($C_{13}H_{19}NO_2$·HCl·0.5H_2O) C, H, N.

2-Methyl-1,3-dioxa-8-(phenylmethyl)-8-azaspiro[4.5]decane (5d). The foregoing diol 7b (42.3 g, 0.19 mol) was converted into the dioxolane free base (17.1 g, 36%) as described for 5a. The hydrogen oxalate salt: mp 116–118 °C; R_f 0.60 in EtOAc/PE (1:1) on alumina plates; MS, m/z 247 (M^+, free base). Anal. ($C_{15}H_{21}NO_2$·$C_2H_2O_4$·0.5H_2O) C, H, N.

2-Methyl-1,3-dioxa-8-azaspiro[4.5]decane Hydrochloride (5b). The *N*-benzyl dioxolane free base 5d (17.0 g, 0.068 mol) was hydrogenated at 50 psi over 20% $Pd(OH)_2$ on C (1.7 g) in EtOH (200 mL) for 36 h, then filtered through Hyflo, and evaporated to give a cream solid (10.25 g, 78%), mp 40–43 °C. The hydrochloride salt: mp 148–150 °C; R_f 0.25 in CH_2Cl_2/MeOH (9:1) on alumina plates; MS, m/z 157 (M^+, free base); ^1H NMR (D_2O) δ 1.41 (3 H, d, J = 5 Hz, $OCHCH_3$), 2.00 (4 H, m, 6- and 10-CH_2), 3.32 (4 H, m, 7- and 9-CH_2), 3.77 and 3.96 (each 1 H, each d, J = 9 Hz, OCH_2), and 5.24 (1 H, q, J = 5 Hz, $OCHCH_3$). Anal. ($C_8H_{15}NO_2$·HCl) C, H, N.

2-Ethyl-8-methyl-1,3-dioxa-8-azaspiro[4.5]decane Hydrochloride (5c). Compound 5c, free base, (4.70 g, 59%) was obtained from propionaldehyde diethyl acetal (25 mL, 0.15 mol) and the diol 7a (6.20 g, 0.043 mol) in the same manner as described for 5a. The hydrochloride salt: mp 98–100 °C; R_f 0.30 in EtOAc on alumina plates; MS, m/z 185 (M^+, free base); ^1H NMR (D_2O) δ 0.94 (3 H, t, J = 8 Hz, CH_2CH_3), 1.72 (2 H, m, CH_2CH_3), 2.05 (4 H, m, 6- and 10-CH_2), 2.89 (3 H, s, NCH_3), 3.26 and 3.50 (each 2 H, each m, 7- and 9-CH_2), 3.78 and 3.90 (each 1 H, each d, J = 9 Hz, OCH_2), 5.09 (1 H, t, J = 5 Hz, $OCHCH_2CH_3$). Anal. ($C_{10}H_{19}NO_2$·HCl) C, H, N.

3-Hydroxy-3-(methoxycarbonyl)-1-(phenylmethyl)-piperidine. 1-(Phenylmethyl)-3-piperidone (8)[13] (30.07 g, 0.16 mol), zinc iodide (0.64 g, 0.002 mol), and trimethylsilyl cyanide (26.6 mL, 0.212 mol) in dry CH_2Cl_2 (80 mL) were heated under reflux for 2 h. The mixture was cooled and evaporated to give the crude *O*-(trimethylsilyl)cyanohydrin 9 (45.15 g), which was poured into concentrated HCl (120 mL) and left at room temperature for 2 days. The solution was evaporated and dried over P_2O_5, yielding the crude 3-hydroxy-1-(phenylmethyl)-3-piperidine carboxylic acid hydrochloride (40.05 g) as a buff-colored solid. This hydroxy acid was dissolved in MeOH (300 mL) at 5 °C, and dry hydrogen chloride gas was bubbled through for 3 h. After 18 h at room temperature, the mixture was evaporated to dryness, and the free base was liberated with aqueous Na_2CO_3 solution followed by CH_2Cl_2 extraction. This crude ester was purified by column chromatography on alumina using EtOAc/PE (1:2) to afford the title compound (4.1 g, 10%) as an orange oil. The hydrogen oxalate salt: mp 56–58 °C; R_f 0.40 in EtOAc/PE (1:1) on alumina plates; MS, m/z 249 (M^+, free base); ^1H NMR (D_2O) δ 1.7–2.2 (4 H, m, 4- and 5-CH_2), 3.10 (1 H, dt, J = 2 Hz and 12 Hz, 6-CH), 3.30 and 3.35 (each 1 H, each d, J = 14 Hz, 2-CH_2), 3.60 (1 H, m, 6-CH), 3.75 (3 H, s, CO_2CH_3), 4.29 and 4.44 (each 1 H, each d, J = 14 Hz, NCH_2 phenyl), and 7.50 (5 H, m, C_6H_5); IR ν_{max} (Nujol) 3600–3000 (OH), 2800–2400 (NH^+), 1730 cm^{-1} (C=O). Anal. ($C_{14}H_{19}NO_3$·$C_2H_2O_4$·H_2O) C, H, N.

3-Hydroxy-3-(hydroxymethyl)-1-(phenylmethyl)-piperidine. The foregoing hydroxy ester (3.9 g, 15.6 mmol) was reduced by using $LiAlH_4$ (0.86 g, 23 mmol) in dry THF (45 mL) as described for 7a. The required diol was obtained (2.84 g, 82%) as a pale yellow oil: MS, m/z 221 (M^+); IR ν_{max} (film) 3600–3100 cm^{-1} (OH).

2-Methyl-7-(phenylmethyl)-1,3-dioxa-7-azaspiro[4.5]decane (10). Compound 10 as a 1:1 mixture of diastereomers was obtained (1.90 g, 64%) as a yellow oil from the above diol (2.67 g, 12 mmol) by using the procedure described for 5a: R_f 0.65 in EtOAc/PE (1:1) on alumina plates; t_R 13.6 and 13.7 min (GC, 100–190 °C gradient); MS, m/z 247 (M^+).

2-Methyl-7-(phenylmethyl)-1,3-dioxa-7-azaspiro[4.5]decane Methiodide. Iodomethane (2.13 mL, 34 mmol) was added to a stirred solution of 10 (1.69 g, 6.8 mmol) in dry acetone (8 mL) and heated under reflux for 3 h. The mixture was evaporated and then triturated with Et_2O/*i*-PA (5:1) to give the methiodide (2.22 g, 84%) as a mixture of isomers: mp 160–164 °C; MS, m/z 246 ($M^+ - CH_3I - H$). Anal. ($C_{16}H_{24}INO_2$) C, H, N.

2,7-Dimethyl-1,3-dioxa-7-azaspiro[4.5]decane Sesquioxalate (4a,b). The above quaternary salt (1.9 g, 4.9 mmol) was hydrogenated at 50 psi in EtOH (20 mL) over 10% Pd on C (0.40 g) for 44 h. The mixture was filtered through Hyflo and then evaporated to give a foam (1.02 g), which was partitioned between CH_2Cl_2 (20 mL) and saturated Na_2CO_3 solution (20 mL). The organic phase was separated, dried, and then evaporated to dryness to give the title compound (0.60 g) as a mixture of diastereomers.

These diastereomers were separated by column chromatography on alumina by elution with EtOAc/PE (1:6) to give diastereomer A (43 mg) and diastereomer B (102 mg).

2,7-Dimethyl-1,3-dioxa-7-azaspiro[4.5]decane sesquioxalate, diastereomer A (45 mg): mp 113–114 °C; R_f 0.57 in EtOAc/PE (1:1) on alumina plates; t_R 2.69 min (free base, GC, 100–115 °C gradient); MS, m/z 171 (M^+, free base); ^1H NMR (D_2O) δ 1.40 (3 H, d, J = 5 Hz, $OCHCH_3$), 1.64 (1 H, dt, J = 6 Hz and 14 Hz, 10-CH), 1.86–2.14 (3 H, m, 9-CH_2 and 10-CH), 2.86 (3 H, s, NCH_3), 2.96 (1 H, dt, J = 4 Hz and 14 Hz, 8-CH), 3.17 and 3.24 (each 1 H, each d, J = 14 Hz, 6-CH_2), 3.30 (1 H, dm, J = 12 Hz, 8-CH), 3.74 and 3.99 (each 1 H, each d, J = 10 Hz, 4-CH_2) and 5.20 (1 H, q, J = 5 Hz, $OCHCH_3$). Anal. ($C_9H_{17}NO_2$·1.5$C_2H_2O_4$) C, H, N.

2,7-Dimethyl-1,3-dioxa-7-azaspiro[4.5]decane sesquioxalate, diastereomer B (74 mg): mp 124–126 °C; R_f 0.53 in EtOAc/PE (1:1) on alumina plates; t_R 2.78 min (free base, GC, 100–115 °C gradient); MS, m/z 171 (M^+, free base); ^1H NMR (D_2O) δ 1.39 (3 H, d, J = 5 Hz, $OCHCH_3$), 1.76 (1 H, dt, J = 6 Hz and 14 Hz, 10-CH), 1.86–2.10 (3 H, m, 9-CH_2 and 10-CH), 2.88 (3 H, s, NCH_3), 2.97 (1 H, dt, J = 4 Hz and 14 Hz, 8-CH), 3.09 and 3.54 (each 1 H, each d, J = 14 Hz, 6-CH_2), 3.48 (1 H, dm, J = 12 Hz, 8-CH), 3.76 and 3.86 (each 1 H, each d, J = 10 Hz, 4-CH_2), and 5.24 (1 H, q, J = 5 Hz, $OCHCH_3$). Anal. ($C_9H_{17}NO_2$·1.5$C_2H_2O_4$) C, H, N.

2-Methyl-1,3-dioxa-7-azaspiro[4.5]decane Hydrogen Oxalate (4c,d). A solution of 10 (5.0 g, 20.2 mmol) in ethanol (40 mL) was hydrogenated over $Pd(OH)_2$ (Pearlman's catalyst, 0.50 g) at 50 psi for 24 h. Filtration through Hyflo and evaporation yielded the title compound (3.0 g, 94%) as a 1:1 mixture of diastereomers. A portion of this material (1.0 g) was purified by column chromatography on alumina, eluting with Et_2O/MeOH (97:3) to give diastereomer A (373 mg) and diastereomer B (220 mg).

2-Methyl-1,3-dioxa-7-azaspiro[4.5]decane hydrogen oxalate, diastereoisomer A (336 mg): mp 106–108 °C; R_f 0.25 in Et_2O/MeOH (95:5) on alumina; t_R 5.21 min (free base, GC, 80 °C isothermal); MS, m/z 157 (M^+, free base); ^1H NMR (D_2O) δ 1.40 (3 H, d, J = 5 Hz, CH_3), 1.71 (1 H, dt, J = 4 Hz and 14 Hz, 10-CH), 1.84–2.08 (3 H, m, 9-CH_2 and 10-CH), 2.98 (1 H, dt, J = 3 Hz and 13 Hz, 8-CH), 3.14 and 3.24 (each 1 H, d, J = 13 Hz, 6-CH_2), 3.36 (1 H, d, J = 13 Hz, 8-CH), 3.70 and 4.02 (each 1 H, d, J = 10 Hz, 4-CH_2), 5.20 (1 H, q, J = 5 Hz, OCH). Anal. ($C_8H_{15}NO_2$·$C_2H_2O_4$) C, H, N.

2-Methyl-1,3-dioxa-7-azaspiro[4.5]decane hydrogen oxalate, diastereomer B (135 mg): mp 125–128 °C; R_f 0.18 in Et_2O/MeOH (95:5) on alumina; t_R 5.35 min (free base, GC, 80 °C isothermal); MS, m/z 157 (M^+, free base); ^1H NMR (D_2O) δ 1.39 (3 H, d, J = 5 Hz, CH_3), 1.80–2.10 (4 H, m, 9-CH_2 and 10-CH_2), 2.98 (1 H, dt, J = 2 Hz and 13 Hz, 8-CH), 3.07 and 3.44 (each 1 H, each d, J = 13 Hz, 6-CH_2), 3.38 (1 H, dd, J = 2 Hz and 13 Hz, 8-CH),

3.76 and 3.85 (each 1 H, each d, J = 9 Hz, 4-CH_2), 5.22 (1 H, q, J = 5 Hz, OCH).

Biochemical Methods. (a) **Binding Studies.** Membranes were prepared from 250–300-g Male Sprague–Dawley rats as described previously[5] and resuspended in 20 mM HEPES Krebs' buffer (pH 7.4) for [^{3}H]-*N*-methylscopolamine binding and 20 mM HEPES buffer (pH 7.4) for [^{3}H]oxotremorine M binding (1 mL total assay volume). Assays were incubated at 30 °C for 60 and 40 min, respectively, and were terminated by filtration through Whatman GF/B and 0.05% polyethylene imine presoaked GF/C filters by using a Brandel cell harvester. Drug displacement curves were assessed by using 0.1 nM [^{3}H]-*N*-methylscopolamine and 3.0 nM [^{3}H]oxotremorine-M.

(b) **Hydrolysis of Inositol Phospholipids.** Tissue slices of rat cerebral cortex (350 × 350 μm) were prepared by using a McIlwain tissue chopper and were washed three times in Krebs–bicarbonate buffer, followed by a 30-min preincubation in the presence of [^{3}H]-*myo*-2-inositol, 2 μCi, (Amersham International, TRK 807 13.8 Ci/mmol) and 10 mM lithium. Tissue slices were subsequently incubated in the presence of muscarinic agonists for 45 min in a 250-μL volume. The reaction was terminated by addition of 940 μL of chloroform/methanol (1:2), and water-soluble inositol monophosphates were isolated by ion-exchange chromatography. The elution methods have previously been described in detail by Brown and colleagues.[19] Radioactivity in the inositol monophosphate fraction was estimated by liquid scintillation spectrometry. All drugs were added in a volume of 10 μL.

(19) Brown, E.; Kendall, D. A.; Nahorski, S. R. *J. Neurochem.* **1984**, *42*, 1379.

Book Reviews

Writing the Laboratory Notebook. By Howard M. Kanare. American Chemical Society, Washington, D.C. 1985. xii + 146 pp. 17 × 24 cm. ISBN 0-8412-0906-5. $19.95.

The aim of this book is to provide laboratory workers and others vitally concerned with recording and use of experimental data with guidelines to record the course of experimentation. Comment on the dustjacket tells us that this is the first text that has "thoroughly covered the myriad aspects of writing and using a laboratory notebook". Mr. Kanare is a chemist and materials scientist but he has structured the book to appeal to a broad scientific readership. Has the author reached his goal? The answer is ambivalent, "Yes" in some ways, "No" in others. The book is concisely and clearly written with frequent useful summaries. Senior and junior personnel will both find much that is instructive in the Reasons, Ethical Aspects, and Patents chapters (1, 3, and 7, respectively).

The Management and Organizing chapters (4 and 5) are also very useful ones. The Ethics and Patents chapters (3 and 7), which should be back-to-back, are critical ones for industrial scientists. Confidentiality is not stressed adequately and the importance of establishing a good working relationship with the patent agent responsible for the area of interest was omitted. The frequently used practice of using separate "idea" notebooks also failed to be mentioned.

Managers charged with responsibilities for setting up organizational record-keeping procedures will find Chapters 4 and 2 (Management and Hardware) valuable.

Scientists outside of chemistry and electronics will not find this book very useful; scope should have been confined to chemists. And chemists must review the book carefully before recommending it to students and junior employees. The transformation of the scientist from "activist"—planning and conducting an experiment—to "judge"—dispassionately weighing the data—and recording both aspects objectively is not stressed. The examples of a well-written notebook (Chapter 6) are not well-chosen. The experiments are trivial and the author advocates a first-person style of writing notes which has led to a wordy, overly personalized series of model pages.

Why the author has failed to reference ACS journals which demand clear, well-written experimentals for publication or other experimental record works, such as "Organic Syntheses", is difficult to understand.

The topic of supplementary records (spectra, chromatography traces, etc.) and how to key them to the notebook is treated too lightly.

The Electronic Notebook (Chapter 8) compares use of a computer terminal for experimental data entry with the traditional handwritten method. Some chemists may have a choice; more typically, the dilemma is how to correlate manual and electronic data records. This chapter is of little aid in solving this problem.

The text is reasonably error-free with high-quality paper and binding and is reasonably priced. The index is adequate while the references vary from appropriate and recent to nonexistent depending on the chapter.

In summary, this text is a useful teaching aid/reference provided that the teacher or supervisor uses discretion.

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Radiation Chemistry. Principles and Applications. Edited by Farhataziz and Michael A. J. Rodgers. VCH, New York. 1987. xii + 641 pp. 16 × 24 cm. ISBN 0-89573-127-4. $95.00.

In its broadest sense radiation chemistry implies the study of chemical changes induced by the interaction of any form of radiation with matter; however it is generally agreed that the scope of the field embraces only those photons (γ and X-rays) and particles (electrons, protons, α and other heavy particles) which possess sufficient energy to induce ionization of the components of a material. Thus it is distinguished from photochemistry, which is concerned with lower energy photons generally capable of producing excited states of molecules but not ions as primary products. In accordance with this, the editors' intent is to provide a broad exposition of radiation chemistry and a description of some scientific areas which depend on it, suitable for students of the field, whether graduate students newly entering the field or practicing scientists in other areas desiring information on radiation chemistry.

The volume consists of 20 chapters, authored by a variety of specialists. The first four chapters are introductory, laying out fundamentals of the interactions of photons and charged particles with matter, the initial products of these interactions, detection methods and instrumentation, and an introduction to the kinetics governing their behavior. The next two chapters are theoretical in nature, covering early phenomena in ionization processes, the structure of ionization tracks and reactions therein. The middle group of chapters develop specific fundamental areas, such as the properties and reactions of the electron and the solvated electron, the radiation chemistry of gases, aqueous and organic liquids, colloidal aggregates, and organic solids. The final several chapters describe the application of radiation chemistry fundamentals to radiation science of polymers, biopolymers, and other biochemicals and the radiation biology of microorganisms and mammalian cells.

Just as the first two-thirds of the book demonstrates the maturity of the field in terms of fundamental studies in simple inorganic and organic systems, so the last several chapters show that there is much current interest in more complex systems, such