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Synthesis and Selective Activity of Cholinergic Agents with Rigid Skeletons. II[1]

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Three types of compound, quinuclidine-3-spiro-2′-dioxolane (A), piperidine-4-spiro-2′-dioxolane (B), and piperidine-4-spiro-2′-(4′-oxodioxolane) (C) and their methiodides were synthesized.

The cholinomimetic activities of these compounds were examined to investigate the relationship between the activities of these compounds, which have rigid skeletons, and those of known muscarinic agents.

Keywords——Cholinergic agents; rigid structure; selective activity; quinuclidinium salts; piperidinium salts; spiro-ketals; muscarinic activity; structure–activity relationship

The authors have reported a hypothetical conformation suitable for acetylcholine binding with muscarinic receptor, obtained by superposing the skeletons of L(+)-muscarine, (+)-*trans*-2*S*-acetoxycyclopropyl-1*S*-trimethylammonium salt, and acetylcholine, and showed that four particular atoms in the skeletons of other known potent muscarinic agonists and antagonists also can coincide spatially with those of acetylcholine. On this basis, the syntheses and cholinomimetic activities of some compounds with semi-rigid structure were reported.[1]

This paper deals with the syntheses and activities of compounds of types A, B, and C (Fig. 1), which were designed on the same basis. Each compound possesses four atoms, (one carbon, one nitrogen, and two oxygen atoms) corresponding to the methyl carbon of the acetyl group, the ammonium nitrogen, and the oxygens in the ester group of acetylcholine, respectively. Figure 1 illustrates molecular shapes in which the atoms are located in the desired positions as nearly as possible. In the compounds of type A, 1-azabicyclo[2.2.2]octane-3-spiro-2′-dioxolanes, the ammonium nitrogen is included in the bridgehead of the rigid quinuclidine skeleton. Another moiety of A corresponding to the acetyl group in acetylcholine (**1**) is deformed to a spiro-ketal structure. Since the ketal (1,3-dioxolane ring) is found in known potent muscarinic agents such as L(+)-*cis*-2*S*-methyl-4*R*-trimethylammoniummethyl-1,3-dioxolane (**2**)[2,3] and its analogs (3—5),[2-4] this moiety is supposed to play a role similar to that of the acetoxy group in acetylcholine. The positions of the substituents on the dioxolane ring in A differ from those of the known dioxolane derivatives (**2**—**5**) ,but type A is regarded as a structure in which the compound **13** is fixed with ethylene bridge. Compound **13** has been synthesized and examined for activity: it showed either low affinity or week contraction.[1] In Fig. 1, the reported muscarinic potencies, expressed in terms of equipotent molar ratio (EPMR) with respect to acetylcholine, are also shown.

The synthetic route to the compounds of type A is shown in Chart 1. 3-Quinuclidone (**6**) was ketalized to give spiro-ketals (**7**—**9**) which were converted to quaternary salts (**10**—**12**) by treatment with methyl iodide in acetone.

Compounds of type B have skeletons in which the spiro-ketal ring in **13** is located at position 4 in the piperidine ring. The relative spatial configurations of the marked C–CH_3, ammonium nitrogen, and two oxygen atoms in B are somewhat different from those of **13**. The

activities of compounds of type B should cast valuable light on the structure–activity relationship.

1-Methyl-4-piperidinone (**14**) was allowed to react with 1,2-glycols in the presence of *p*-toluenesulfonic acid in benzene using a Dean–Stark apparatus. The resulting spiro-ketals (**15**—**18**) were quaternized to give the corresponding methiodides (**19**—**22**) (Chart 2).

Compound **31**, a 4-oxo-ketal (Fig. 1), has been synthesized and found to show low potency.[1] To investigate the pharmacological effect of the shift of the ketal ring, compounds of type C were

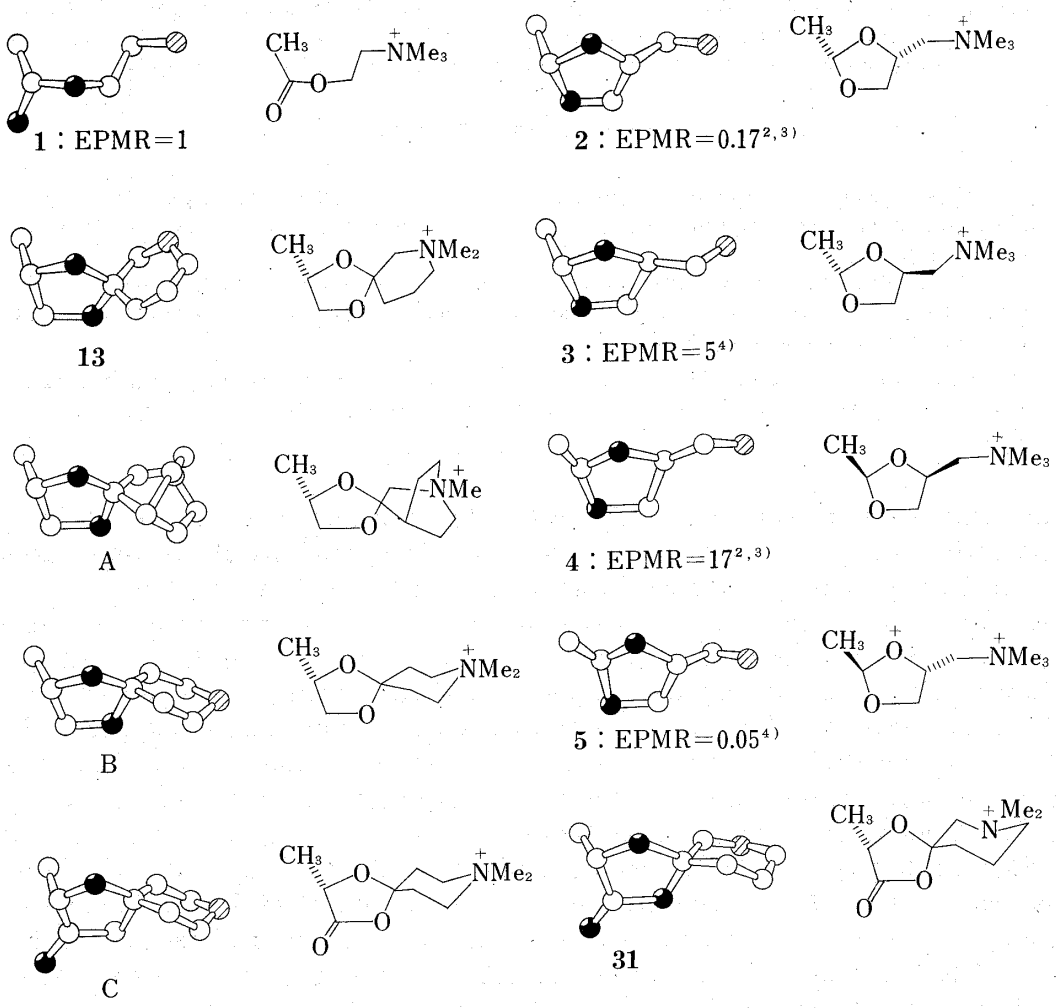
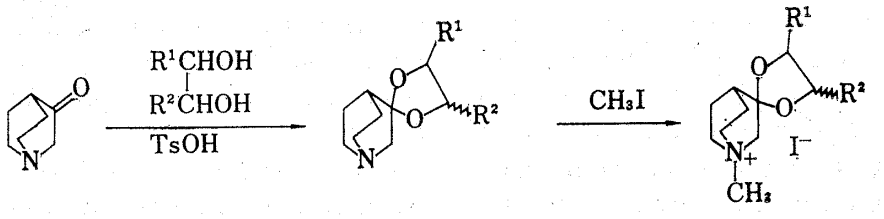


Fig. 1. Structures and Molecular Shapes of Acetylcholine (**1**), Known Potent Muscarinic Dioxolane Derivatives (**2**—**5**), Piperidinium-3-spiro-ketal (**13**), and Designed Compounds (A, B, and C)

○, carbon; ◍, nitrogen; ◕, oxygen.

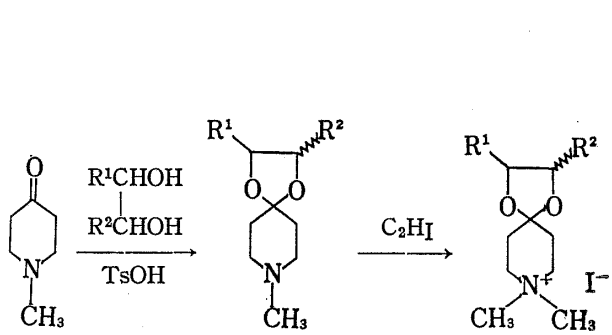


		R¹	R²
6	**7:**	H	H
	8:	CH_3	H
	9:	CH_3	CH_3

	R¹	R²
10:	H	H
11:	CH_3	H
12:	CH_3	CH_3

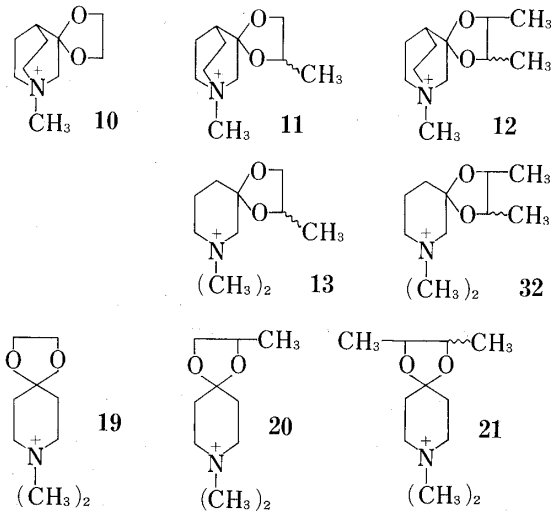
Chart 1

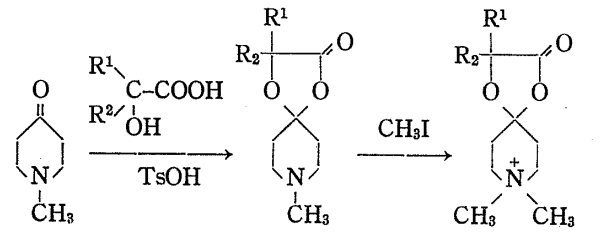
prepared (Chart 3). The piperidinone (**14**) was refluxed with α-hydroxy acids in the presence of an acid catalyst with azeotropic removal of water to give the spiro compounds (**23**—**26**), which were converted to the corresponding quaternary salts (**27**—**30**). The phenyl-substituted analogs, **22**, **29**, and **30** were expected to show muscarinic antagonistic action because a number of antagonists, such as atropine, benactizine, or piperidolate, have structures in which hydrogen(s) of C–CH_3 in acetylcholine is replaced by one or two phenyl groups.



	R^1	R^2		R^1	R^2
14					
15:	H	H	**19:**	H	H
16:	CH_3	H	**20:**	CH_3	H
17:	CH_3	CH_3	**21:**	CH_3	CH_3
18:	C_6H_5	H	**22:**	C_6H_5	H

Chart 2





	R^1	R^2		R^1	R^2
14					
23:	CH_3	H	**27:**	CH_3	H
24:	CH_3	CH_3	**28:**	CH_3	CH_3
25:	C_6H_5	H	**29:**	C_6H_5	H
26:	C_6H_5	C_6H_5	**30:**	C_6H_5	C_6H_5

Chart 3

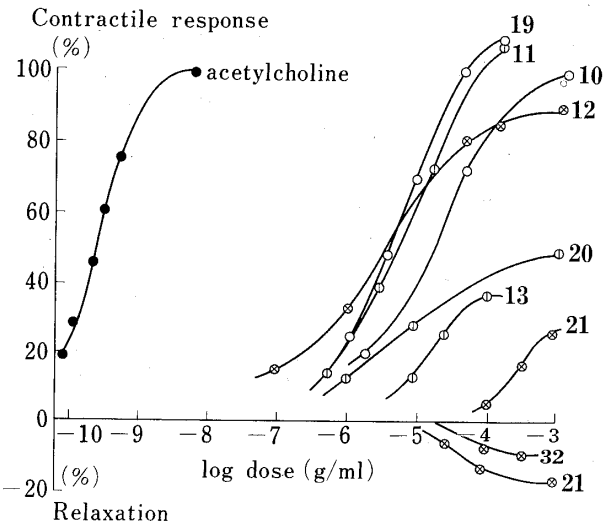


Fig. 2. Dose-response Curves of Spiro-ketals of Quinuclidinium and Piperidinium Salts (Guinea-pig Ileum)

Pharmacology and Discussion

The cholinomimetic activities of the synthesized compounds were examined by the Magnus method. The changes in tonus of guinea-pig ileum suspended in Locke solution (NaCl, 9.0 g; KCl, 0.42 g; $CaCl_2$, 0.24 g; $NaHCO_3$; 1.0 g; glucose, 1.0 g; pH 7.2—7.4) were isotonically recorded at 25±1°C. The average values of 5 experiments were plotted against dose.

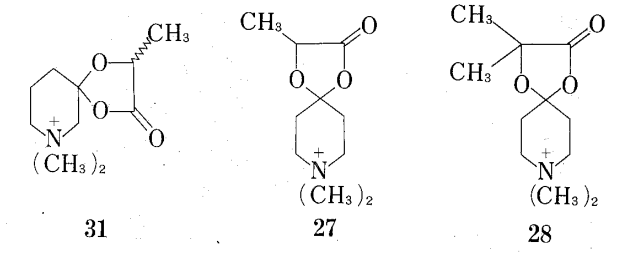
Figure 2 shows the contractile responses of compounds **10**—**12** and **19**—**21**, based on the maximum contraction of acetylcholine as 100%, as a standard.

The quinuclidinium salts of type A, **10**—**12**, exhibited strong contractions, comparable to that of acetylcholine, but at high doses. Among them, the monomethyl compound, **11**, showed

the highest contraction and the responses decreased in the order of **11**>**10**>**12**. These results are compared in the figure with the activities of the piperidinium analogs, **13** and **32**, which were reported previously.[1] Compounds **13** exhibited only 30% of the contraction of acetylcholine and compound **32** showed about 10% relaxation. The large increases in the contractile response on going from **13** to **11** and from **32** to **12** may be regarded as due to the fixing of the piperidine ring with the ethylene bridge. The contraction activities of 4-spiro-ketal piperidinium salts fall in the order of **19**>**20**>**21**.[5] The effect of the change from the monomethyl to the dimethyl derivatives, **20** to **21**, resembles that in going from **13** to **32**, but this is not the case in the quinuclidinium salts (**11** to **12**). These results suggest that the binding modes of the 4-spiro-ketals (**19**—**21**) on the receptor resemble those of the 3-spiro-ketals (**13**—**32**) rather than those of the quinuclidinium salts (**10**—**12**). Thus, the binding mode of quinuclidinium salts may be different from that of piperidinium salts. The requirement for higher doses of the compounds tested as compared with that of acetylcholine might be a result of crowding around the ammonium nitrogens. A similar effect was observed in the relationship between 3-acetoxypiperidinium salt and acetylcholine.[1] The marked decrease in contraction upon dimethyl substitution of parent compounds such as **12**, **21**, and **32** suggests the presence of a strict steric requirement in the region complementary to the methyl group in the acetylcholine receptor.[4]

Compounds **27** and **28** have 4-spiro-ketal -lactone structures and their activities, together with that of the positional isomer **31** are shown as dose-response curves in Fig. 3. The shift of the methylated ketal-lactone from position 3 to 4 results a considerable increase in either affinity or contraction. A contraction decreasing effect similar to that discussed above was also observed in this series.

The phenyl-substituted derivatives, **22**, **29**, and **30**, showed no acetylcholine-like activity but had antagonistic activity. Figure 4, the inhibition of the contraction of the muscle caused by acetylcholine is plotted against the dose, based on the action of atropine taken as 100%.



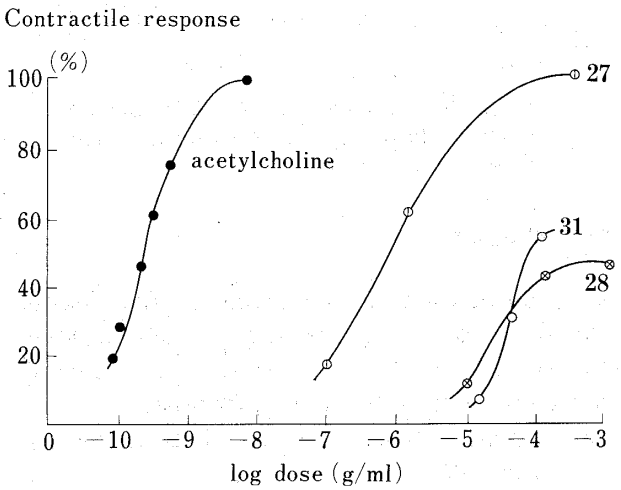
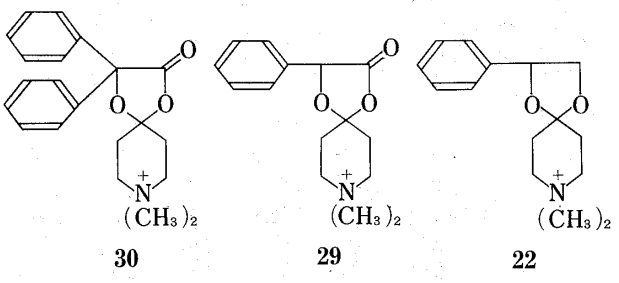


Fig. 3. Dose-response Curves of Spiro-oxodioxolans of Piperidinium Salts (Guinea-pig Ileum)



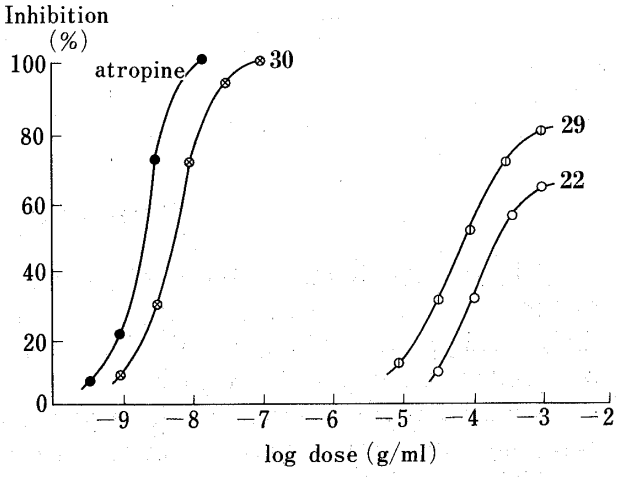


Fig. 4. Dose-inhibition Curves of Phenyl-substituted Spiro-ketals of Piperidinium Salts

Among the compounds tested, **30** showed a potent antagonistic effect comparable to that of atropine. Therefore, it appears that substitution with a phenyl group near the C-methyl corresponding to that of acetylcholine results in atropine-like activity.

Experimental

1-Azabicyclo[2.2.2]octane-3-spiro-2′-dioxolane (**7**)——A mixture of 3-quinuclidone hydrochloride (1.54 g, 9.5 mmol), ethylene glycol (12 ml, 0.19 mol), *p*-toluenesulfonic acid (0.2 g, 1.1 mmol), and toluene (25 ml) was refluxed for 3 h in a Dean–Stark apparatus. The mixture was cooled, neutralized with 20% aqueous K_2CO_3 and extracted with benzene. The organic layer was washed with H_2O and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the residue was distilled to collect the desired fraction, bp_{20} 113—117°C (0.68 g, 42%). The product was directly used for the quaternization. NMR ($CDCl_3$) δ: 1.4—2.2 (5H, m, CH_2CH_2N and CH), 2.6—3.2 (6H, m, CH_2N), 3.92 (4H, s, CH_2O).

1-Azabicyclo[2.2.2]octane-3-spiro-2′-dioxolane Methiodide (**10**)——Compound **7** (0.67 g) was dissolved in acetone (1 ml), and methyl iodide (0.25 ml) was added. The mixture was allowed to stand overnight in a refrigerator, then the separated crystals were filtered off and recrystallized from EtOH to give the pure product, mp 217—218°C (0.98 g, 80%). *Anal.* Calcd for $C_{10}H_{18}INO_2$: C, 38.60; H, 5.83; N, 4.50; I, 40.78. Found: C, 38.77; H, 5.94; N, 4.40; I, 40.89.

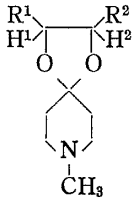
1-Azabicyclo[2.2.2]octane-3-spiro-2′-(4′-methyldioxolane) (**8**)——3-Quinuclidone hydrochloride (1.54 g, 9.4 mmol), propylene glycol (8 ml), and *p*-toluenesulfonic acid (1.94 g, 10.2 mmol) were mixed with toluene (25 ml) and the whole was refluxed for 5.5 h. The reaction mixture was treated as described for the preparation of **7**. An oil (1.25 g, 72%), bp_{19} 117—119°C, was obtained. It was directly used for the next step. NMR ($CDCl_3$) δ: 1.3 (3H, d, J=5 Hz, CH_3), 1.45—2.20 (5H, m, CH_2CH_2N and CH), 2.80—3.3 (6H, m, CH_2N), 3.3—3.5 (1H, m, CH_3CH), 3.85—4.4 (2H, m, CH_2CHCH_3).

1-Azabicyclo[2.2.2]octane-3-spiro-2′-(4′-methyldioxolane) Methiodide (**11**)——Compound **8** (1.25 g) was quaternized by a method similar to that described for **10**, giving the pure product as crystals, mp 163—165°C

Table I. Reaction of 1-Methyl-4-piperidone (**14**) with Diols

diols a: $HOCH_2CH_2OH$
b: $HOCH(CH_3)CH_2OH$
c: $HOCH(CH_3)CH(CH_3)OH$
d: $HOCH(C_6H_5)CH_2OH$

products

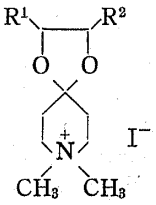


15: $R^1=R^2=H$
16: $R^1=CH_3$, $R^2=H$
17: $R^1=R^2=CH_3$
18: $R^1=C_6H_5$, $R^2=H$

Diol	**14** (g)	Solvent (C_6H_6, ml)	Time (h)	Product	Yield g(%)	bp(°C) (mmHg)
a	0.94	25	3	**15**	0.34 (26.0)	94—98 (31)
b	2.00	30	25	**16**	0.85 (30.0)	90 (14)
c	2.00	30	5	**17**	2.05 (65.4)	90—91 (14)
d	2.00	30	35	**18**	3.33 (80.8)	169—170 (12)

Diol	NMR ($CDCl_3$) R^1	R^2	H^1	H^2	1-CH_3	3,5-H	2,6-H
a	——— 3.96 (s) ———				2.31 (s)	1.6—1.9 (m)	2.3—2.7 (m)
b	1.27 (d) J=7 Hz	3.9—4.5 (m)	3.45 (t) J=7 Hz	3.9—4.5 (m)	2.30 (s)	1.6—1.8 (m)	2.3—2.7 (m)
c	1.2—1.4 (m)	—	3.4—3.8 (m)	—	2.30 (m)	1.7—1.9 (m)	2.3—2.7 (m)
d	7.34 (s)	5.07 (dd) J=8.6 Hz	3.69 (t) J=8 Hz	4.31 (dd) J=8.6 Hz	2.32 (s)	1.7—2.1 (m)	2.4—2.7 (m)

TABLE II. 1-Methylpiperidine-4-spiro-2′-dioxolane Methiodides

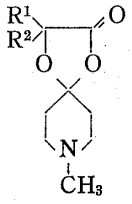


19: $R^1=R^2=H$
20: $R^1=CH_3$, $R^2=H$
21: $R^1=R^2=CH_3$
22: $R^1=C_6H_5$, $R_2=H$

Compound	Yield (%)	mp (°C)	Formula	Analysis Calcd (Found) C	H	N	I
19	91.9	271—272	$C_9H_{18}INO_2$	36.13 (36.31	6.02 6.24	4.68 4.69	42.46 42.49)
20	80.3	232—234	$C_{10}H_{20}INO_2$	38.35 (38.42	6.44 6.61	4.47 4.44	40.52 40.66)
21	86.9	242—243	$C_{11}H_{22}INO_2$	40.38 (40.14	6.78 6.82	4.28 4.25	38.79 39.03)
22	99.0	224—225	$C_{15}H_{22}INO_2$	48.00 (47.85	5.87 5.98	3.73 3.61	33.87 34.06)

TABLE III. Reaction of 1-Methyl-4-piperidone (**14**) with α-Hydroxy Acids

α-hydroxy acids
a: $CH_3CH(OH)COOH$
b: $(CH_3)_2C(OH)COOH$
c: $C_6H_5C(OH)COOH$
d: $(C_6H_5)_2C(OH)COOH$



23: $R^1=CH_3$, $R^2=H$
24: $R^1=R^2=CH_3$
25: $R^1=C_6H_5$, $R^2=H$
26: $R^1=R^2=C_6H_5$

Acid	**14** (g)	Solvent (C_6H_6, ml)	Time (h)	Product	Yield g(%)	bp(mp) (°C) (mmHg)
a	2.0	30	17.5	**23**	0.48 (15.5)	116—117 (11)
b	1.0	30	6.5	**24**	1.44 (85.7)	160—165 (11)
c	1.5	30	13.5	**25**	1.88 (61.2)	166—169 (4)
d	1.0	30	8.5	**26**	2.40 (86.2)	mp 126—127

Acid	NMR ($CDCl_3$) R^1	R^2	1-CH_3	3,5-H	2,6-H	IR (cm^{-1}) (νco)
a	1.48 (d) J=6.5 Hz	4.46 (q) J=6.5 Hz	2.32 (s)	1.7—2.1 (m)	2.4—2.7 (m)	1800
b	— 1.49 — (s)		2.32 (s)	1.8—2.1 (m)	2.4—2.7 (m)	1795
c	7.40 (s)	5.38 (s)	2.33 (s)	1.9—2.2 (m)	2.4—2.8 (m)	1790
d	—7.1—7.7— (m)		2.31 (s)	1.8—2.1 (s)	2.3—2.8 (m)	1790

(1.3 g, 60%). *Anal.* Calcd for $C_{12}H_{20}INO_2$: C, 40.63; H, 6.20; N, 4.31; I, 39.02. Found: C, 40.35; H, 6.43; N, 4.40; I, 38.66.

1-Azabicyclo[2.2.2]octane-3-spiro-2′-(4′,5′-dimethyldioxolane) (9)——3-Quinuclidone hydrochloride (1.54 g, 9.5 mmol), 2,3-butanediol (8.36 ml, 95 mmol), and *p*-toluenesulfonic acid (0.88 g, 4.6 mmol) were mixed with toluene (25 ml) and the whole was refluxed for 21 h. The reaction mixture was treated as described for **7** and **8** to give an oil, bp_{20} 115—120°C (1.43 g, 76.5%). The product was used for the next step without further purification. NMR ($CDCl_3$) δ: 1.0—1.5 (6H, m, CH_3), 1.55—2.25 (5H, m, CH_2CH_2N and CH), 2.6—3.2 (6H, m, CH_2N), 3.4—3.8 (2H, m, CH_3CH).

1-Azabicyclo[2.2.2]octane-3-spiro-2′-(4′,5′-dimethyldioxolane) Methiodide (12)——The tertiary amine (**9**) (1.43 g) was treated with methyl iodide as described for **10** and **11** to obtain crude crystals, which were recrystallized from acetone, mp 194—195°C (1.31 g, 53%). *Anal.* Calcd for $C_{12}H_{22}INO_2$: C, 42.49; H, 6.54; N, 4.13; I, 37.41. Found: C, 42.52; H, 6.62; N, 4.27; I, 37.35.

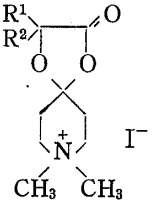
1-Methylpiperidine-4-spiro-2′-dioxolanes (15—18) (General Procedure)——1-Methyl-4-piperidone (**14**) and a diol were refluxed with benzene in a Dean–Stark apparatus in the presence of *p*-toluenesulfonic acid until the spot of **14** disappeared on a thin-layer chromatogram of the reaction mixture. The reaction mixture was neutralized with 10% K_2CO_3 and extracted with benzene. The organic layer was washed with saturated NaCl solution and dried over Na_2SO_4. After the solvent had been removed by distillation, the residue was distilled under reduced preseure. The data for individual compounds are listed in Table I.

1-Methylpiperidine-4-spiro-2′-dioxolane Methiodides (19—22) (General Method)——The ketal-amine (**15—18**) and methyl iodide were mixed in acetone and the solution was allowed to stand overnight. The separated crystals were filtered off and recrystallized from EtOH. The data for the products are listed in Table II.

1-Methylpiperidine-4-spiro-2′-(4′-oxodioxolane) (23—26) (General Method)——1-Methyl-4-piperidone (**14**) and an α-hydroxy acid reacted in the same way as described for **15—18**; the reaction conditions and data are listed in Table III.

1-Methylperidine-4-spiro-2′-(4′-oxodioxolane) Methiodides (27—30)——A tertiary amine (**23—26**) was treated with methyl iodide as described for **19—22**. The data for the products are given in Table IV.

Table IV. 1-Methylpiperidine-4-spiro-2′-(4′-oxodioxolane) Methiodide (**27—30**)



27: $R^1=CH_3$, $R^2=H$
28: $R^1=R^2=CH_3$
29: $R^1=C_6H_5$, $R_2=H$
30: $R^1=R^2=C_6H_5$

Compound	Yield (%)	mp (°C)	Formula	Analysis Calcd (Found)			
				C	H	N	I
27	74.7	244—245	$C_{10}H_{18}INO_3$	36.71 (36.88	5.55 5.47	4.28 4.32	38.79 38.97)
28	84.1	243—245	$C_{11}H_{20}INO_3$	38.72 (38.69	5.87 6.04	4.11 3.98	37.22 37.52)
29	45.5	267—268	$C_{15}H_{20}INO_3$	46.28 (46.30	5.14 5.02	3.60 3.44	32.63 32.57)
30	90.0	281—282	$C_{21}H_{24}INO_3$	54.20 (54.41	5.20 5.52	3.01 3.22	27.27 27.55)

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References and Notes

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5) Two curves are shown for compound **21** due to individual differences.