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Studies on Antitumor Substances. XIV.[1] Syntheses of Bisquarternary Ammonium Salts and the Reaction with Nucleophiles

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Several bisquarternary ammonium salts were synthesized and tested as to their antitumor activity. However, none of them exhibited any effective activity. Additionally, alkylation of nucleophiles with quarternary ammonium salts, dimethylaminomethyl *p*-tolyl ether methiodide and ethylene bis (N,N-diethyl-N-methylammonium iodide), was examined. Dimethylaminomethyl *p*-tolyl ether methiodide reacted successfully with nucleophiles, such as amine, thiol and alcohol, to give the expected compounds in which *p*-tolyloxymethyl group was introduced to the nucleophilic compounds. On the other hand, ethylene bis (N,N-diethyl-N-methylammonium iodide) reacted with thiophenol and diethyl malonate to give the methylated compounds, methyl phenyl sulfide and diethyl malonate respectively.

It has been well known that Myleran (I, $n=4$)[3,4] possess carcinostatic activities. Recently Hayashi, *et al.*[5,6] have reported that thiosulfonates (II), which is the isoster of Myleran, also inhibited the growth of Ehrlich's solid tumor in mice. The antitumor effect of Myleran has been suggested to be attributed to the alkylating property, because Myleran reacted with the sulfhydryl group in cystein *in vitro* to form the tetrahydrothiophene derivative.[7] While, thiosulfonate (II) was revealed to possess reactivities[8] different from those of Myleran. If some compounds have alkylating properties, the compounds may be expected to provide effective chemotherapeutic drugs against tumors. Thus, in the relation with Myleran (I) and thiosulfonate (II), polymethylene bis(quarternary ammonium salts) (III) were attempted

$CH_3SO_2O(CH_2)_nOSO_2CH_3$, I $\quad CH_3SO_2S(CH_2)_nSSO_2CH_3$, II $\quad R-\overset{+}{N}(CH_2)_n\overset{+}{N}-R$ (R, R, R, R), III

to prepare and tested as to antitumor effects of them. Alkylation with quarternary ammonium[9] salts has hitherto been widely investigated and applied in certain special fields, for example the synthesis of amino acid.[10,11] This paper is concerned with the syntheses of polymethylene bis(quarternary ammonium salts) and their reactivities toward some nucleophiles.

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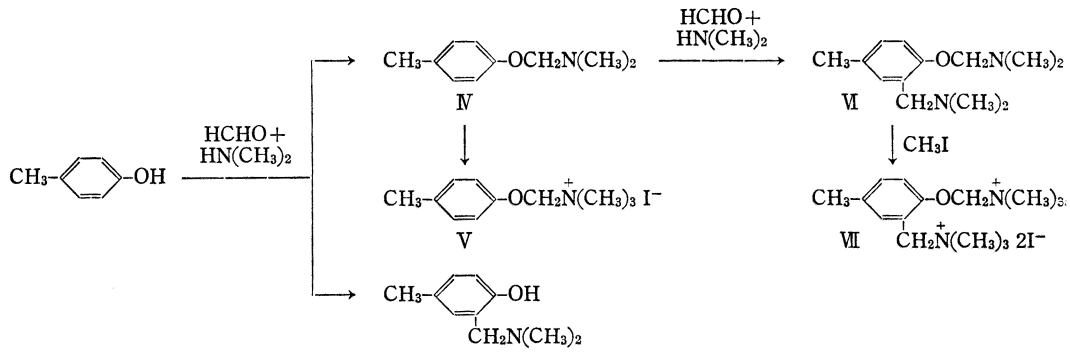
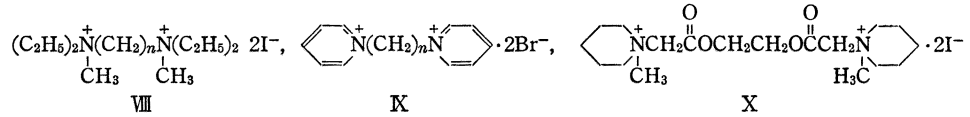
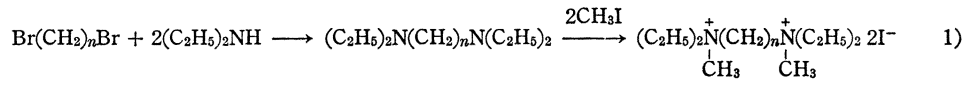
Syntheses of Quarternary Ammonium Salt

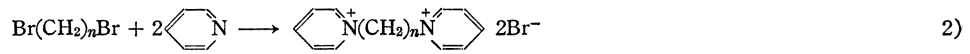
First, in order to examine the alkylation with the etheric Mannich base, dimethylaminomethyl *p*-tolyl ether methiodide was synthesized. A number of Mannich reaction of phenols[12–16] have generally resulted in the introduction of the dimethylaminomethyl group in the *ortho* or *para* position of the benzene nucleus. While, Delépine[13] has reported that Mannich reaction of phenol gave a mixture of 2-dimethylaminomethylphenol and dimethylaminomethyl phenyl ether. Mannich reaction of *p*-cresol[16] has also been reported to give 4-methyl-2-dimethylaminomethylphenol. However, Mannich reaction of *p*-cresol in the similar reaction condition was found to give dimethylaminomethyl *p*-tolyl ether (IV) in 63% yield, which was readily converted to the corresponding methiodide (V). Dimethylaminomethyl *p*-tolyl ether (IV) was confirmed by the negative ferric chloride test and the infrared (IR) spectrum in which the absorption assignable to the vinyl group was exhibited at 1270 cm^{-1}, but no absorption due to a hydroxyl group was observed. Additional Mannich reaction of dimethylaminomethyl *p*-tolyl ether (IV) gave dimethylaminomethyl 4-methyl-2-dimethylaminomethylphenyl ether (VI) in 70% yield, which was also easily converted to the corresponding methiodide (VII) by the usual method.

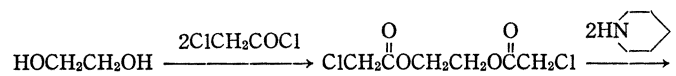


Next, five polymethylene bis(quarternary ammonium salts), polymethylene bis(N,N-diethyl-N-methylammonium iodide) (VIII, n=2,3), polymethylene bis(pyridinium bromide) (IX, n=2,3) and ethylene bis(piperidinoacetate methiodide) (X), were successfully synthesized respectively by the following three methods.









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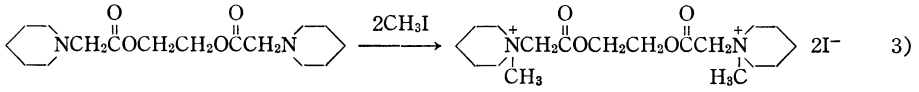
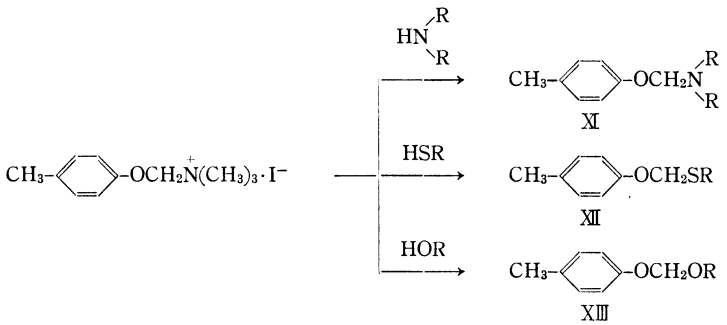


Alkylene dibromide was heated with an excess of diethylamine to give polymethylene bis(N,N-diethylamine), followed by treating with methyl iodide by the usual method to give the corresponding methiodide (VIII) in about 55% overall yields (method 1). Heating of alkylene dibromide with an excess of pyridine under reflux gave analogously polymethylene bis(pyridinium bromide) (IX) in 70—80% yields (method 2). Ethylene bis(piperidinoacetate methiodide) (X) was prepared through three steps from ethylene glycol in 27% overall yield (method 3). Heating of ethylene glycol with an excess of chloroacetyl chloride gave ethylene bischloroacetate, which was converted to ethylene bispiperidinoacetate by treating with an excess of piperidine at room temperature. By usual method, ethylene bispiperidinoacetate was readily converted to the corresponding methiodide in good yield.

Reaction of Quarternary Ammonium Salt with Nucleophile

Although a number of alkylation with Mannich base,[9,17,18] which involved the quarternary ammonium salt, have been known, little has been reported relating to the alkylation with etheric Monnich base, in which the ether bond is involved in the α-position of the methylene group. Thus, dimethylaminomethyl *p*-tolyl ether methiodide (V) was allowed to react with nucleophiles, such as amine, thiol, alcohol, phenol and active methylene compound. Heating of dimethylaminomethyl *p*-tolyl ether methiodide (V) with amines, such as piperidine, morpholine and piperazine, in anhydrous ethanol in the presence of sodium ethoxide gave the corresponding amine exchange products (XI), in which *p*-tolyloxymethyl group was introduced to the amines, in 30—40% yields. Heating of dimethylaminomethyl *p*-tolyl ether methiodide (V) with thiols, such as iso-propanethiol, *n*-butanethiol and *t*-butanethiol, also afforded the expected sulfides (XII), in which *p*-tolyloxymethyl group was involved, in 30—65% yields. Analogously, heating of dimethylaminomethyl *p*-tolyl ether



methiodide (V) with alcohols, such as methanol and ethanol, also resulted in the formation of the corresponding methylenedioxy compounds (XIII) in about 35% yields. Unfortunately, the reactions with phenols and active methylene compounds were, however, unsuccessful and small amounts of the material were recovered. These compounds obtained were summarized in Table I.

Next, the reaction between ethylene bis(N,N-diethyl-N-methylammonium iodide) (VIII, $n=2$) and some nucleophiles was attempted. Alkylation with bisquarternary ammonium salt has never reported in the literature. In the alkylation of nucleophiles with quarternary ammonium salts, the most bulky group is generally introduced in the nucleophilic com-

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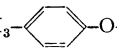
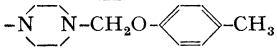
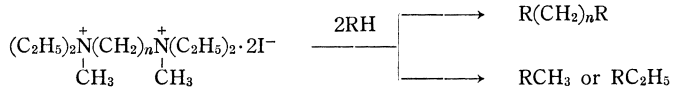
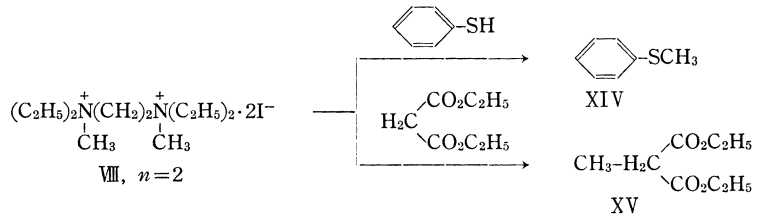
Table I. CH_3–  –O–CH_2–R

No.	R	bp or mp (°C/mm) (°C)	Yield	Aanlysis (%) Calcd. C	H	N	Found C	H	N
XI-a	 •HCl	191.5—192	31.1	64.59	8.28	5.79	64.15	8.16	6.05
XI-b	 •HCl	190—190.5	36.0	59.13	7.39	5.90	59.11	7.37	5.79
XI-c		222—222.5	38.0	71.61	8.11	8.35	71.55	7.87	8.45
XII-a	–S–$CH(CH_3)_2$	101—103/2	47.5	67.37	8.16		67.00	7.86	
XII-b	–S–$C(CH_3)_3$	113/3	31.3	68.57	8.63		68.16	8.39	
XII-c	–S–C_4H_9	143/4	63.5	68.57	8.63		67.92	8.29	
XIII-a	–O–CH_3	85/3	38.0	71.02	7.95		70.64	8.07	
XIII-b	–O–C_2H_5	78/4.5	32.7	72.26	8.49		72.40	8.71	

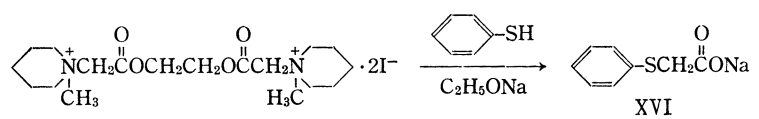
pounds, as can be seen in the reaction described above. In polymethylene bis(N,N-diethyl-N-methylammonium iodide) (VIII), however, there is no difference in the bulkiness of the four rests adjacent to the nitrogen atom. Therefore, two different orientations of the alkyl-



ation may be possible. The reaction was carried out by heating in anhydrous ethanol in the presence of sodium ethoxide. Morpholine, piperidine, phenol, *p*-nitrophenol, thiophenol, cyanoacetamide and diethyl malonate were employed as nucleophiles. Among them, thiophenol and diethyl malonate reacted successfully with ethylene bis(N,N-diethyl-N-methylammonium iodide) (VIII, $n=2$) to give methyl phenyl sulfide (XIV) and diethyl methylmalonate (XV) in 32% and 12% yields, respectively, though all of another cases were un-



successful to obtain the expected compounds. Contrary to the expectation, the alkylation of nucleophiles with polymethylene bis(pyridinium bromide) (IX) resulted in failure. On the other hand, heating of thiophenol with ethylene bis(piperidinoacetate methiodide) (X) under the similar condition gave sodium phenylthioacetate (XVI) in 10% yield, without formation of any expected methyl phenyl sulfide. Probably, it would be attributed to the increasing positive charge in the methylene group, owing to the adjacent carbonyl group.



The antitumor effect of bisquarternary ammonium salts synthesized was examined by the screening test using Hela cells. However, none of them exhibited the effective antitumor activity.

Experimental

Dimethylaminomethyl *p*-Tolyl Ether Methiodide (V)——To a mixture of 27 g of *p*-cresol and 70 g of 40% dimethylamine aqueous solution was added dropwise with stirring 57 g of 37% formaline during 1 hr below 20°. The mixture was heated for an additional 2 hr at 80°, and on cooling the mixture was extracted with ether. The extracts were dried over Na_2SO_4 and evaporated. The residue was distilled under reduced pressure to give 29 g (71%) of dimethylaminomethyl *p*-tolyl ether boiling at 72°/1.5 mm. IR ν_{max}^{film} cm^{-1}: 1270 (-C=C-O-C-). To a solution of 29 g of dimethylaminomethyl *p*-tolyl ether in acetone was added dropwise with stirring 30 g of methyl iodide during 1 hr at room temperature. The precipitates deposited were filtered and recrystallized from H_2O to give 34 g (62.9%) of colorless colums melting at 149—150°. *Anal.* Calcd. for $C_{11}H_{18}ONI$: C, 42.71; H, 5.83; N, 4.56. Found: C, 42.20; H, 6.22; N, 4.86.

Dimethylaminomethyl 2-Dimethylaminomethyl-4-methylphenyl Ether Methiodide (VII)——A solution of 16.3 g of paraformaldehyde and 51.3 g of 40% dimethylamine aqueous solution in a small amount of EtOH was added to a solution of 30 g of dimethylaminomethyl *p*-tolyl ether in EtOH. The solution was stood for 1 hr at room temperature and then heated for an additional 2 hr under reflux. After removal of EtOH, the solution was extracted with ether. The extracts were washed with 5% NaOH aqueous solution and then H_2O, dried over Na_2SO_4 and evaporated. The residue was distilled under reduced pressure to give 28 g (69.3%) of dimethylaminomethyl 2-dimethylaminomethyl-4-methylphenyl ether boiling at 113°/1.5 mm. IR ν_{max}^{film} cm^{-1}: 1260 (-C=C-O-C-). *Anal.* Calcd. for $C_{13}H_{22}ON_2$: C, 70.23; H, 9.97; N, 12.60. Found: C, 70.46; H, 9.80; N, 12.14. To a solution of 28 g of dimethylaminomethyl 2-dimethylaminomethyl-4-methylphenyl ether in acetone was added dropwise with stirring 40 g of methyliodide during 1 hr at room temperature. The precipitates deposited were recrystallized from 95% EtOH to give 51.0 g (79.7%) of colorless needles meldles melting at 227—227.5°. *Anal.* Calcd. for $C_{15}H_{28}ON_2I$: C, 35.60; H, 5.58; N, 5.54. Found: C, 35.47; H, 5.70; N, 5.58.

Ethylene Bis(N,N-diethyl-N-methylammonium Iodide) (VIII, $n=2$)——To 42 g of diethylamine was added slowly with stirring under reflux 30 g of ethylene dibromide. After cooling, 100 ml of 12% NaOH aqueous solution was added to the solution and the mixture was extracted with benzene. The extracts were dried over Na_2SO_4 and evaporated. The residue was distilled under reduced pressure to give 24 g of bis(diethylamino)ethane boiling at 99—100°/42 mm. This compound was converted to the methiodide by the procedure discribed above. Recrystallization from MeOH gave 42 g (57.5% overall yield) of colorless needles melting at 226.5—227°. *Anal.* Calcd. for $C_{12}H_{30}N_2I_2$: C, 31.59; H, 6.63; N, 6.14. Found: C, 31.71; H, 6.35; N, 6.20.

Trimethylene Bis(N,N-diethyl-N-methylammonium Iodide) (VIII, $n=3$)——By the same procedure as described above, 65 g of diethylamine was treated with 40 g of trimethylene dibromide to give 27 g of bis(diethylamino)propane boiling at 69—72°/3.5 mm, which was converted to the methiodide. Recrystallization from 95% EtOH gave 52 g (56.5%) of right yellow needles melting at 245—246°. *Anal.* Calcd. for $C_{13}H_{32}N_2I_2$: C, 33.12; H, 6.86; N, 5.95. Found: C, 33.41; H, 6.56; N, 5.38.

Ethylene Bis(pyridinium Bromide) (IX, $n=2$)——To 50 g of pyridine was added with stirring under reflux 50 g of ethylene dibromide. After cooling, the precipitates deposited were filtered and recrystallized from MeOH to give 65 g (70.6%) of colorless needles melting at 284—284.5°. *Anal.* Calcd. for $C_{12}H_{14}N_2Br_2$: C, 41.65; H, 4.08; N, 8.10. Found: C, 41.53; H, 3.99; N, 7.83.

Trimethylene Bis(pyridinium Bromide) (IX, $n=3$)——By the same procedure as described above, 50 g of pyridine was treated with 55 g of trimethylene dibromide to give 78 g (79.6%) of colorless needles melting at 236—237°. *Anal.* Calcd. for $C_{13}H_{16}N_2Br_2$: C, 43.36; H, 4.48; N, 7.78. Found: C, 42.87; H, 4.38; N, 7.66.

Ethylene Bis(piperidinoacetate Methiodide) (X)——To 98 g of choroacetyl chloride was added dropwise under reflux during 1 hr 30 g of ethylene glycol, and the solution was refluxed for an additional 2 hr until evolution of HCl disappeared. After removal of an excess of chloroacetyl chloride, distillation of the residue gave 64 g of ethylene dichloroacetate boiling at 124—126°/2 mm, which was added with stirring to 204 g of piperidine at room temperature. The resulted mixture was washed with CCl_4 to remove piperidine hydrochloride and the washings were distilled under reduced pressure to give 48 g of ethylene bispiperidinoacetate boiling at 181—183°/0.05 mm, which was converted to the methiodide by the usual method. Recrystallization from 95% EtOH gave 74 g (26.7% overall yield) of colorless needles melting at 195.5°. *Anal.* Calcd. for $C_{18}H_{32}O_4N_2I_2$: C, 36.38; H, 5.43; N, 4.71. Found: C, 36.54; H, 5.70; N, 4.90.

Reaction of Dimethylaminomethyl *p*-Tolyl Ether Methiodide (V) with Nucleophiles——a) With Amine: A solution of 0.033 mole of sodium ethoxide and 0.033 mole of amine in EtOH was heated for 20 min under reflux. To the solution was added 0.033 mole of dimethylaminomethyl *p*-tolyl ether methiodide, and the mixture was heated for 20 hr under reflux. After removal of EtOH, benzene was added to the residue and

the resulted precipitates were filtered off. Dry HCl was passed through the filtrate. Resulting aminomethyl *p*-tolyl ether HCl precipitated was washed with acetone and recrystallized from EtOH or $CHCl_3$. Details of the data were summarized in Table I.

b) With Thiol: To a solution of 0.066 mole of sodium ethoxide and 0.066 mole of thiol in EtOH was added with stirring 0.066 mole of dimethylaminomethyl *p*-tolyl ether methiodide, and the solution was heated for 20 hr under reflux. After removal of the solvent, the residue was poured into H_2O and extracted with ether. The extracts were dried over Na_2SO_4 and evaporated. The residue was distilled under reduced pressure to give alkylthiomethyl *p*-tolyl ether. Details of the data were shown in Table I.

c) With Alcohol: A solution of 0.033 mole of dimethylaminomethyl *p*-tolyl ether methiodide in anhydrous EtOH containing 0.033 mole of sodium ethoxide was heated for 20 hr under reflux. After removal of the solvent, the residue was poured into H_2O and extracted with ether. The extracts were dried over Na_2SO_4 and evaporated. The residue was distilled under reduced pressure to give alkoxymethyl *p*-tolyl ether. Details of the data were summarized in Table I.

Reaction of Ethylene Bis(N,N-diethyl-N-methylammonium Iodide) (VIII, $n=2$) with Thiophenol——A solution of sodilum ethoxide (prepared from 0.5 g of Na) and 8.7 g of thiophenol in EtOH was heated for 1 hr under reflux. To the solution was added 18 g of ethylene bis(N,N-diethyl-N-methylammonium iodide), and the mixture was heated for 20 hr under reflux. After removal of the solvent, the residue was poured into H_2O and extracted with ether. The extracts were dried over Na_2SO_4 and evaporated. The residue was distilled under recuced pressure to give 3.1 g (32%) of methyl phenyl sulfide boiling at 67—68°/11 mm, which was identified with the authentic sample. *Anal.* Calcd. for C_7H_8S: C, 66.93; H, 6.45. Found: C, 66.61; H, 6.59.

Reaction of Ethylene Bis(N,N-diethyl-N-methylammonium Iodide) (VIII, $n=2$) with Diethyl Malonate——After a solution of sodium ethoxide (prepared from 1 g of Na) and 7.0 g of diethyl malonate was refluxed for 1 hr, 10 g of ethylene bis(N,N-diethyl-N-methylammonium iodide) was added to the solution, and the mixture was heated for 20 hr under reflux. The mixture was poured into H_2O and extracted with ether. The extracts were dried over Na_2SO_4 and evaporated. Distillation of the residue gave 0.5 g (12%) of diethyl methylmalonate boiling at 84.5°/2 mm, which was identified with the authentic sample. *Anal.* Calcd. for $C_8H_{14}O_4$: C, 55.38; H, 7.57. Found: C, 54.90; H, 7.74.

Reaction of Ethylene Bis(piperidinoacetate Methiodide) (X) with Thiophenol——A solution of sodium ethoxide (prepared from 0.78 g of Na) and 3.7 g of thiophenol in EtOH was refluxed for 1 hr. To the solution was added 10 g of ethylene bis(piperidinoacetate methiodide), and the mixture was heated for 20 hr under reflux. The precipitates deposited on cooling were recrystallized from 50% EtOH to give 0.3 g (10.5%) of colorless needles of sodium phenylthioacetate melting at 302—303°. *Anal.* Calcd. for $C_8H_7O_2SNa$: C, 50.53; H, 3.68. Found: C, 50.51; H, 3.72.

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