[Chem. Pharm. Bull. 15(12)1828~1840(1967)]

UDC 615.511 : 615.778.475 : 547.587.11

233. Kiichiro Kakemi, Takaichi Arita, Shikifumi Kitazawa, and Yoshihisa Sagawa*1 : Studies on the Pharmaceutical Potentiation of Drugs. II.*2 *p*-Aminosalicylic Acid Derivatives.

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Nine series of ω-substituted PAS alkyl esters, namely, ω-chloroalkyl, ω-aminoalkyl, ω-diethylaminoalkyl, ω-phenylethylaminoalkyl, ω-morpholinoalkyl, ω-pyrrolidinylalkyl, ω-piperidinoalkyl *p*-aminosalicylates and alkylene bis-*p*-aminosalicylates in which the alkyl length varied from ethyl to decyl were synthesized and their evaluations were made with testing their tuberculostatic activities and with measuring their physicochemical properties such as partition coefficients and degrees of protein binding that might considerably influence biological effects of these substances when administered in animal body. These synthesized derivatives have activities as same extent as the parent compound and are more lipid soluble and some of them are less protein bound than PAS. From these observations, it is considered that some of the defects of the parent compound are improved by these chemical modifications. Relationship between structures and these characteristics are also revealed.

(Received May 30, 1966)

That the intrinsic tuberculostatic activities and the physicochemical characteristics, such as partition coefficients and degrees of binding to bovine serum albumin, of ω-substituted alkyl esters of *p*-aminosalicylic acid (PAS) are considerably influenced by both the chemical constitution of ω-substituents and the length of alkyl chain have been demonstrated in this laboratory.

The purpose of the present investigation is to extend the substituents on the end of the alkyl chain and to scrutinize their influences on their intrinsic antitubercular activities and physico-chemical properties above mentioned.

It is widely recognized that the introduction of chlorine atoms in the molecule increase the antibacterial and antifungal activities[1] and, at the same time, Smith[2] and Clark[3] reported that compounds with halogen atoms have tendencies to prolong their duration time. From the viewpoint of drug latentiation, compounds that have two PAS moieties in one molecule would be expected to have considerable biological interests.

On the other hand, Gaddum[4] reported that amino group and its related substituents have great effects on biological activities of the parent compounds.

In this paper ω-chloroalkyl, ω-aminoalkyl, ω-diethylaminoalkyl, ω-phenylethylaminoalkyl, ω-diphenylaminoalkyl, ω-morpholinoalkyl, ω-pyrrolidinylalkyl, ω-piperidinoalkyl *p*-aminosalicylates and alkylene bis-*p*-aminosalicylates with alkyl length systematically from ethyl to decyl were synthesized, and their antitubercular activities against *Myc. tuberculosis* H37Rv, partition coefficients and degrees of protein binding ratios were determined.

Of these derivatives ω-chloroethyl *p*-aminosalicylate,[5,6] ω-diethylaminoethyl and propyl *p*-aminosalicylates,[7,8] and ω-piperidinoethyl and propyl *p*-aminosalicylates[7,8]

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*2 Part I : This Bulletin, **15**, 1819 (1967).

1) G. M. Dyson, P. May : "Chemistry of synthetic drugs" (1959), Longmans, Green Co., Ltd.
2) M. Smith : "Mode of action of drugs on cells" (1933). Arnold Co., Ltd.
3) W. D. Clark : "Enzymes and Drug action" (1962). Churchill Co., Ltd.
4) J. H. Gaddum : "Pharmacology," 5th ed. (1959). Oxford Co., Ltd.
5) W. Grimme : Chem. Ber., **84**, 734 (1951); German Patent 842944.
6) H. von Euler : Arkiv für Kemi., **2**, 297 (1950).
7) R. O. Clinton : J. Am. Chem. Soc., **73**, 3674 (1951).
8) W. Grimme, H. Schmitz : Chem. Ber., **84**, 734 (1951).

were known in literatures, but no systematical investigations for the physico-chemical properties have been done.

In the studies of preparations of homologous series of derivatives of ω-chloroalkyl esters of PAS, the method, given by Grimme and Euler in the case of ω-chloroethyl ester, was found to be applicable to the syntheses of esters of rather short chains. Since, however, the condensation with alcohols of more than six carbon atoms yielded only resin and seemed to be difficult for further purifications, it was thought advisable to investigate another procedure for the preparations. Indirect condensations were attempted, that were, *p*-nitrosalicylic acid (PNS) and the corresponding alkylene chlorohydrine were condensed and then reduced to amino derivatives using stannous chloride and hydrochloric acid, and found the major products were the objective compounds. Further details of these syntheses will be mentioned in experimental.

Most of alkylene chlorohydrines, used as materials for these preparations, were obtained by the method of Coleman.[9] Butylene chlorohydrine was obtained by the method of Starr[10] and ethylene chlorohydrine was obtained commercially.

In the studies of preparations of homologous series of ω-substituted aminoalkyl esters, the synthesizing methods proposed by Clinton for ω-diethylaminoethyl and propyl derivatives which applied the condensation of ω-diethylaminoalkyl chloride and nitrosalicylic acid, was found not applicable to the syntheses of these esters of longer alkyl chains. The difficulties encountered in applying the Clinton's method were purification of the reaction mixtures especially on separations of the materials and the products, furthermore, resulted esters of nitro compounds were found to be somewhat unstable. Some experimental works were carried out in the determination of a suitable procedure for the preparation of these esters.

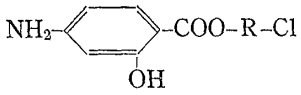
In the cases of the syntheses of ω-diethylaminoalkyl, ω-phenylethylaminoalkyl and ω-diphenylaminoalkyl esters, direct esterifications using PAS and corresponding alcohols in the presence of conc. sulfuric acid were tried but gave unsatisfactory results. Best results were obtained from the condensations of these substituted amines or sodium salt of these amines and ω-chloroalkyl *p*-nitrosalicylates in the presence or without sodium iodide. Since these amines were easily soluble in water, purifications of these reaction mixtures could be achieved only by slight washing with water. Reduction of these nitro compounds to the corresponding amino derivatives were then proceeded without further purification by catalytic hydrogenation using palladium charcoal as catalyst. Diethylaminoethyl and propyl esters of this series were oily substances when they were synthesized, as Clinton reported, but on standing in a desiccator for weeks, they crystallized having melting points of 32° and 38°, and could be recrystallized from small amount of ethanol. Their melting points of monohydrochlorides were 225° and 231° and were identified as Clinton mentioned.

In the cases of ω-morpholinoalkyl, ω-pyrrolidinoalkyl and ω-piperidinoalkyl esters, the direct esterification with keeping the temperature at 90° gave somewhat satisfactory results. Further details of this reaction will be mentioned in experimental. ω-Morpholinoalkyl alcohols and other ω-substituted alkyl alcohols used in the above procedure were synthesized by condensation of ω-chloroalkyl alcohol and morpholine and/or pyrrolidine and/or piperidine in the presence of sodium iodide in ethanol as solvent.

In the preparations of homologous series of ω-aminoalkyl esters, direct condensation of PAS with ω-aminoalkyl alcohols with the temperature not above 90° gave very poor yield. Although with poor yield and many difficulties in the course of the purifications, the indirect condensation seemed to be the best way to obtain these substances. The

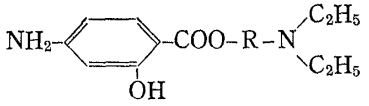
9) W. Coleman : J. Am. Chem. Soc., **66**, 1821 (1944).
10) D. Starr : Org. Syntheses, Coll., **II**, 571 (1943).

Table I. ω-Chloroalkyl p-Aminosalicylates



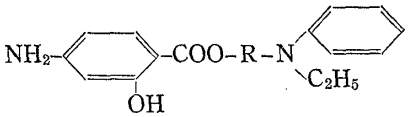
R	Appearances	m.p. & b.p. (mm. Hg, °C)	Recrystal. solvent	Yield (%)	Formula	Analyses (%) Calcd. C	H	N	Found C	H	N
C_2H_4	colorless needles	118	MeOH+H_2O	80.2	$C_9H_{10}O_3NCl$	50.13	4.68	6.50	50.08	4.77	6.39
C_3H_6	colorless needles	83	MeOH	53.0	$C_{10}H_{12}O_3NCl$	52.29	5.27	6.10	52.30	5.51	6.40
C_4H_8	pale-yellow plates	102	MeOH	85.7	$C_{11}H_{14}O_3NCl$	54.21	5.79	5.75	53.99	5.82	5.72
C_5H_{10}	colorless needles	66	MeOH	60.0	$C_{12}H_{16}O_3NCl$	55.93	6.26	5.43	55.87	6.32	5.31
C_6H_{12}	colorless needles	60	EtOH	63.1	$C_{13}H_{18}O_3NCl$	57.46	6.68	5.16	57.42	6.38	5.51
C_7H_{14}	colorless plates	57	EtOH	62.0	$C_{14}H_{20}O_3NCl$	58.84	7.06	4.90	58.52	7.32	4.69
C_8H_{16}	colorless needles	72	EtOH	58.0	$C_{15}H_{22}O_3NCl$	60.09	7.40	4.67	60.31	7.23	4.51
C_9H_{18}	pale-yellow oil	250(0.1)		32.5	$C_{16}H_{24}O_3NCl$	61.24	7.71	4.46	61.51	7.82	4.61
$C_{10}H_{20}$	pale-yellow oil	290(0.1)		38.6	$C_{17}H_{26}O_3NCl$	62.29	8.00	4.27	62.51	8.30	4.51

Table II. ω-Diethylaminoalkyl p-Aminosalicylates



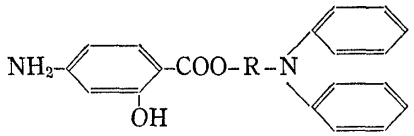
R	Appearances	m.p. (°C)	Recrystal. solvent	Yield (%)	Formula	Analyses (%) Calcd. C	H	N	Found C	H	N
C_2H_4	pale-yellow needles	32	EtOH	25.3	$C_{13}H_{20}O_3N_2$	61.88	7.99	11.10	61.59	7.82	11.32
C_3H_6	pale-yellow needles	38	EtOH	23.6	$C_{14}H_{22}O_3N_2$	63.13	8.33	10.52	63.42	8.29	10.52
C_4H_8	pale-yellow needles	53	EtOH	27.4	$C_{15}H_{24}O_3N_2$	64.26	8.63	9.99	64.21	8.72	10.15
C_5H_{10}	pale-yellow plates	65	EtOH	55.1	$C_{16}H_{26}O_3N_2$	65.28	8.90	9.52	65.71	8.92	9.52
C_6H_{12}	pale-yellow plates	58	EtOH	30.8	$C_{17}H_{28}O_3N_2$	66.20	9.15	9.08	66.31	9.41	9.23
C_7H_{14}	pale-yellow plates	42.5	EtOH	21.1	$C_{18}H_{30}O_3N_2$	67.05	9.38	8.68	67.32	9.51	8.73
C_8H_{16}	pale-yellow plates	47	EtOH	11.9	$C_{19}H_{32}O_3N_2$	67.82	9.59	8.33	67.79	9.71	8.51
C_9H_{18}	pale-yellow plates	53	EtOH	15.4	$C_{20}H_{34}O_3N_2$	68.50	9.78	7.99	68.72	9.51	7.98
$C_{10}H_{20}$	pale-yellow plates	64.5	AcOEt	13.4	$C_{21}H_{36}O_3N_2$	69.19	9.96	7.69	69.31	9.69	7.39

Table III. ω-Phenylethylaminoalkyl p-Aminosalicylates



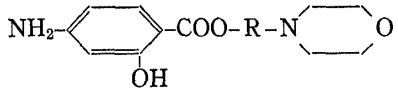
R	Appearances	m.p. (°C)	Recrystal. solvent	Yield (%)	Formula	Analyses (%) Calcd. C	H	N	Found C	H	N
C_2H_4	colorless powder	93	MeOH	10.3	$C_{17}H_{20}O_3N_2$	67.98	6.71	9.33	67.92	6.81	9.32
C_3H_6	pale-yellow powder	84.5	MeOH	21.4	$C_{18}H_{22}O_3N_2$	68.77	7.05	8.91	68.59	7.32	8.95
C_4H_8	pale-yellow prisms	87	MeOH	15.9	$C_{19}H_{24}O_3N_2$	69.49	7.37	8.53	69.51	7.63	8.71
C_5H_{10}	pale-yellow powder	67	EtOH	18.0	$C_{20}H_{26}O_3N_2$	70.15	7.65	8.18	70.32	7.81	8.41
C_6H_{12}	pale-yellow powder	61	MeOH	11.5	$C_{21}H_{28}O_3N_2$	70.76	7.92	7.86	70.51	7.96	7.59
C_7H_{14}	pale-yellow prisms	58.5	EtOH	9.5	$C_{22}H_{30}O_3N_2$	71.32	8.16	7.56	71.31	8.41	7.59
C_8H_{16}	pale-yellow powder	73.5	AcOEt	12.5	$C_{23}H_{32}O_3N_2$	71.84	8.39	7.29	71.59	8.51	7.32
C_9H_{18}	pale-yellow prisms	85	AcOEt	7.8	$C_{24}H_{34}O_3N_2$	72.33	8.60	7.03	72.31	8.65	7.31
$C_{10}H_{20}$	pale-yellow powder	92	AcOEt	12.9	$C_{25}H_{36}O_3N_2$	72.78	8.80	6.79	72.73	8.99	6.81

Table Ⅳ. ω-Diphenylaminoalkyl *p*-Aminosalicylates



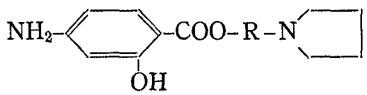
R	Appearances	m.p. (°C)	Recrystal. solvent	Yield (%)	Formula	Analyses (%) Calcd. C	H	N	Found C	H	N
C_2H_4	colorless powder	175	AcOEt	21.7	$C_{21}H_{20}O_3N_2$	72.39	5.79	8.04	72.51	5.91	8.31
C_3H_6	pale-yellow prisms	137	AcOEt	23.1	$C_{22}H_{22}O_3N_2$	72.91	6.12	7.73	72.93	6.35	7.59
C_4H_8	pale-yellow prisms	152	AcOEt	25.3	$C_{23}H_{24}O_3N_2$	73.38	6.43	7.44	73.31	6.52	7.32
C_5H_{10}	pale-yellow plates	123	EtOH	38.5	$C_{24}H_{26}O_3N_2$	73.82	6.71	7.18	73.59	6.82	7.32
C_6H_{12}	pale-yellow plates	112	EtOH	21.9	$C_{25}H_{28}O_3N_2$	74.23	6.98	6.93	74.51	6.99	7.21
C_7H_{14}	pale-yellow prisms	93	AcOEt	17.3	$C_{26}H_{30}O_3N_2$	74.61	7.23	6.69	74.49	7.51	6.81
C_8H_{16}	colorless powder	102.5	EtOH	15.1	$C_{27}H_{32}O_3N_2$	74.97	7.46	6.48	74.59	7.32	6.51
C_9H_{18}	colorless powder	108	EtOH	12.5	$C_{28}H_{34}O_3N_2$	75.30	7.67	6.27	75.60	7.89	6.32
$C_{10}H_{20}$	colorless powder	113	EtOH	12.9	$C_{29}H_{36}O_3N_2$	75.62	7.88	6.08	75.59	7.59	6.31

Table Ⅴ. ω-Morpholinoalkyl *p*-Aminosalicylates



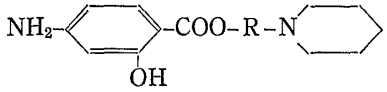
R	Appearances	m.p. & b.p. (mm. Hg, °C)	Recrystal. solvent	Yield (%)	Formula	Analyses (%) Calcd. C	H	N	Found C	H	N
C_2H_4	colorless powder	87	MeOH	23.5	$C_{13}H_{18}O_4N_2$	58.63	6.81	10.52	58.61	6.85	10.72
C_3H_6	colorless powder	97	MeOH	19.7	$C_{14}H_{20}O_4N_2$	59.98	7.19	9.99	59.91	7.32	10.03
C_4H_8	colorless powder	90.5	MeOH	21.3	$C_{15}H_{22}O_4N_2$	61.20	7.53	9.52	61.49	7.41	9.81
C_5H_{10}	colorless powder	107	MeOH	13.9	$C_{16}H_{24}O_4N_2$	62.31	7.85	9.09	62.59	7.94	9.51
C_6H_{12}	colorless powder	95	MeOH	19.8	$C_{17}H_{26}O_4N_2$	63.33	8.13	8.69	63.62	8.49	8.39
C_7H_{14}	colorless oil	165(6)		31.7	$C_{18}H_{28}O_4N_2$	64.26	8.39	8.33	64.51	8.41	8.52
C_8H_{16}	colorless powder	72	MeOH	18.3	$C_{19}H_{30}O_4N_2$	65.11	8.63	7.99	65.32	8.77	7.91
C_9H_{18}	colorless powder	92.5	MeOH	20.7	$C_{20}H_{32}O_4N_2$	65.90	8.85	7.69	65.75	8.67	7.53
$C_{10}H_{20}$	colorless powder	98	MeOH	23.2	$C_{21}H_{34}O_4N_2$	66.63	9.05	7.40	66.39	9.31	7.56

Table Ⅵ. ω-Pyrrolidinylalkyl *p*-Aminosalicylates



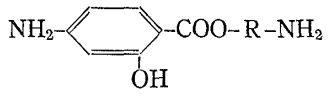
R	Appearances	m.p. & b.p. (mm. Hg, °C)	Recrystal. solvent	Yield (%)	Formula	Analyses (%) Calcd. C	H	N	Found C	H	N
C_2H_4	colorless powder	65	EtOH	32.7	$C_{13}H_{18}O_3N_2$	62.38	7.25	11.19	62.52	7.31	11 01
C_3H_6	colorless powder	70	MeOH	36.9	$C_{14}H_{20}O_3N_2$	63.61	7.63	10.60	63.54	7.89	10.82
C_4H_8	colorless powder	77	AcOEt	27.2	$C_{15}H_{22}O_3N_2$	64.72	7.97	10.07	64.56	7.63	10.32
C_5H_{10}	colorless prisms	89	AcOEt	21.3	$C_{16}H_{24}O_3N_2$	65.72	8.27	9.58	65.99	8.57	9.51
C_6H_{12}	colorless powder	78	AcOEt	20.8	$C_{17}H_{26}O_3N_2$	66.64	8.55	9.14	66.72	8.88	9.32
C_7H_{14}	colorless oil	152(6)		28.5	$C_{18}H_{28}O_3N_2$	67.47	8.81	8.74	67.51	8.98	8.52
C_8H_{16}	colorless powder	66	EtOH	21.6	$C_{19}H_{30}O_3N_2$	68.23	9.04	8.38	68.51	9.32	8.65
C_9H_{18}	colorless powder	75	EtOH	13.7	$C_{20}H_{32}O_3N_2$	68.93	9.26	8.04	68.79	9.35	8.31
$C_{10}H_{20}$	colorless powder	92	EtOH	15.7	$C_{21}H_{34}O_3N_2$	69.58	9.45	7.73	69.43	9.75	7.62

Table Ⅶ. ω-Piperidinoalkyl *p*-Aminosalicylates



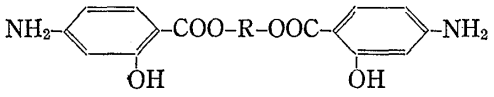
R	Appearances	m.p. & b.p. (mm. Hg, ℃)	Recrystal. solvent	Yield (%)	Formula	Analyses (%) Calcd. C	H	N	Found C	H	N
C_2H_4	colorless powder	79	EtOH	21.6	$C_{14}H_{20}O_3N_2$	63.61	7.63	10.60	63.59	7.77	10.53
C_3H_6	colorless powder	80	EtOH	13.8	$C_{15}H_{22}O_3N_2$	64.72	7.97	10.07	64.59	7.63	10.32
C_4H_8	colorless prisms	88.5	EtOH	25.7	$C_{16}H_{24}O_3N_2$	65.72	8.27	9.58	65.83	8.53	9.51
C_5H_{10}	colorless powder	72	EtOH	23.6	$C_{17}H_{26}O_3N_2$	66.64	8.55	9.14	66.59	8.57	9.33
C_6H_{12}	colorless powder	65	EtOH	26.7	$C_{18}H_{28}O_3N_2$	67.47	8.81	8.74	67.56	8.51	8.59
C_7H_{14}	colorless oil	150(6)		27.3	$C_{19}H_{30}O_3N_2$	68.23	9.04	8.38	68.51	9.21	8.43
C_8H_{16}	colorless powder	70	EtOH	13.2	$C_{20}H_{32}O_3N_2$	68.93	9.26	8.04	68.69	9.51	8.23
C_9H_{18}	colorless powder	53	EtOH	21.6	$C_{21}H_{34}O_3N_2$	69.58	9.54	7.73	69.59	9.63	7.63
$C_{10}H_{20}$	colorless powder	72	EtOH	18.9	$C_{22}H_{36}O_3N_2$	70.17	9.64	7.44	70.51	9.59	7.43

Table Ⅷ. ω-Aminoalkyl *p*-Aminosalicylates



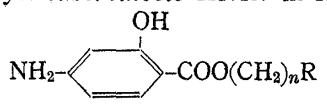
R	Appearances	m.p. & b.p. (mm. Hg, ℃)	Recrystal. solvent	Yield (%)	Formula	Analyses (%) Calcd. C	H	N	Found C	H	N
C_2H_4	colorless needles	82	AcOEt	10.2	$C_9H_{12}O_3N_2$	55.09	6.17	14.28	54.91	6.32	14.31
C_3H_6	colorless needles	53	AcOEt	9.7	$C_{10}H_{14}O_3N_2$	57.13	6.71	13.33	57.01	6.82	13.42
C_4H_8	colorless plates	69	AcOEt	8.3	$C_{11}H_{16}O_3N_2$	58.91	7.19	12.49	58.62	7.31	12.51
C_5H_{10}	colorless prisms	49	AcOEt	10.3	$C_{12}H_{18}O_3N_2$	60.48	7.61	11.76	60.31	7.85	11.49
C_6H_{12}	colorless prisms	38	AcOEt	7.5	$C_{13}H_{20}O_3N_2$	61.88	7.99	11.10	61.59	7.98	11.21
C_7H_{14}	colorless oil	210(0.5)		10.0	$C_{14}H_{22}O_3N_2$	63.13	8.33	10.52	63.50	8.51	10.42
C_8H_{16}	pale-yellow oil	215(0.5)		9.5	$C_{15}H_{24}O_3N_2$	64.26	8.63	9.99	64.51	8.42	10.05
C_9H_{18}	pale-yellow prisms	35	AcOEt	7.5	$C_{16}H_{26}O_3N_2$	65.28	8.90	9.52	65.31	8.99	9.51
$C_{10}H_{20}$	colorless prisms	48	AcOEt	10.5	$C_{17}H_{28}O_3N_2$	66.20	9.15	9.08	66.32	9.02	9.32

Table Ⅸ. Alkylene bis(*p*-Aminosalicylates)



R	Appearances	m.p. (℃)	Recrystal. solvent	Yield (%)	Formula	Analyses (%) Calcd. C	H	N	Found C	H	N
C_2H_4	colorless plates	172.5	EtOH+H_2O	75.3	$C_{16}H_{16}O_6N_2$	57.83	4.85	8.43	56.59	4.91	8.56
C_3H_6	pale-yellow plates	132	EtOH+H_2O	53.6	$C_{17}H_{18}O_6N_2$	58.95	5.24	8.09	58.92	5.32	8.31
C_4H_8	pale-yellow needles	160	EtOH+Me_2CO	45.0	$C_{18}H_{20}O_6N_2$	59.99	5.59	7.77	59.93	5.72	7.58
C_5H_{10}	colorless needles	120	EtOH+Me_2CO	55.0	$C_{19}H_{22}O_6N_2$	60.95	5.92	7.48	60.91	5.69	7.52
C_6H_{12}	colorless plates	110	EtOH	50.5	$C_{20}H_{24}O_6N_2$	61.84	6.23	7.21	61.70	6.01	7.20
C_7H_{14}	pale-yellow needles	93	EtOH	52.1	$C_{21}H_{26}O_6N_2$	62.67	6.51	6.96	62.74	6.38	6.79
C_8H_{16}	pale-yellow needles	99	EtOH	58.5	$C_{22}H_{28}O_6N_2$	63.44	6.78	6.73	63.75	6.92	6.51
C_9H_{18}	colorless prisms	105	EtOH	62.3	$C_{23}H_{30}O_6N_2$	64.17	7.02	6.51	64.32	7.40	6.80
$C_{10}H_{20}$	pale-yellow prisms	113.5	EtOH	55.5	$C_{24}H_{32}O_6N_2$	64.84	7.26	6.30	64.81	7.32	6.51

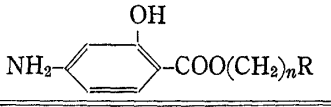
Table X. Minimum Inhibitory Concentrations of the Derivatives on the Growth of *Myc. tuberculosis* H37Rv in Kirchner Medium



($\times 10^{-5}M$/L.)

n	R		
	Amino	bis	Chloro
2	2.55	0.15	2.33
3	1.20	0.72	1.09
4	0.56	0.35	0.26
5	0.26	0.17	0.06
6	0.25	0.04	0.05
7	0.12	0.03	0.11
8	0.06	0.07	0.10
9	0.21	0.07	0.20
10	0.41	0.14	0.38
PAS	0.01		

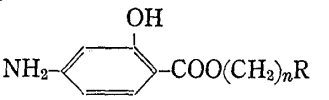
Table XI. Minimum Inhibitory Concentrations of the Derivatives on the Growth of *Myc. tuberculosis* H37Rv in Kirchner Medium



($\times 10^{-5}M$/L.)

n	R		
	Diethylamino	Phenylethylamino	Diphenylamino
2	0.25	0.10	0.07
3	0.11	0.05	0.012
4	0.10	0.02	0.010
5	0.08	0.01	0.0003
6	0.08	0.007	0.001
7	0.03	0.003	0.047
8	0.07	0.003	0.048
9	0.07	0.025	0.09
10	0.07	0.07	0.18
PAS	0.06		

Table XII. Minimum Inhibitory Concentrations of the Derivatives on the Growth of *Myc. tuberculosis* H37Rv in Kirchner Medium



($\times 10^{-5}M$/L.)

n	R		
	Morpholino	Pyrrolidinyl	Piperidino
2	0.5	1	0.5
3	0.25	0.25	0.125
4	0.25	0.25	0.125
5	1	0.25	0.25
6	1	0.25	0.25
7	4	4	4
8	2	0.5	0.06
9	4	2	4
10	0.5	2	0.5
PAS	0.25		

method carried out in these syntheses was condensation of PNS and ω-aminoalkyl alcohol in the presence of conc. sulfuric acid and the temperature was raised up to 110° for eight to fifteen hours, corresponding to the length of alkyl chain. The products were reduced immediately without further purifications. Further details of the syntheses will be mentioned in experimental. Aminoalkyl alcohols, used in the syntheses, were obtained by the method of Coleman, but ethyl and propyl of these homologous were obtained commercially.

On the preparations of the homologous series of alkylene bis-*p*-aminosalicylates, possibilitis of these syntheses were encountered in the investigations on preparations of ω-hydroxyalkyl esters, which were reported previously.[11] In this case the investigation was attracted only on the ratio of the materials, they were alkylene glycol and *p*-nitrosalicyloyl chloride, and reaction time, and found that the optimum ratio of the materials were 1:2.5, regardless to the length of alkyl chain, but reaction time increase with increasing of the length of alkyl chain such as four hours for ethylene and ten hours for decamethylene derivatives. Further details of these syntheses will be mentioned in experimental.

The esters thus obtained are listed in Table I~IX.

The determinations of the *in vitro* intrinsic tuberculostatic activities of these derivatives were carried out by measuring the minimum inhibitory concentrations against the virluent H37Rv strain of *Myc. tuberculosis* by two fold serial dilution method in Kirchner medium. The results are listed in Table X~XII.

Characteristic features of association of intrinsic antitubercular activities with the length of alkyl chain can also be seen in these homologous series of the derivatives except series of ω-morpholinoalkyl, ω-pyrrolidinolalkyl and ω-piperidinoalkyl esters. Compounds with short alkyl length exhibit little of action, but as the chains are lengthened, the activities are also increased, reaching a maximum, and further lengthening bring decrease in the activities.

As shown in Tables, in the series of alkylene bis derivatives, heptamethylene bis-*p*-aminosalicylate had a maximum of $0.03\times10^{-5}M$/L, and in the series of ω-diethylaminoalkyl esters, ω-diethylaminoheptyl *p*-aminosalicylate had a maximum activity of $0.03\times10^{-5}M$/L., almost the same activity as that of PAS, and in the series of ω-phenylethylaminoalkyl esters, ω-phenylethylaminoheptyl ester had a maximum of $0.003\times10^{-5}M$/L. of concentration, about ten times of PAS, and in the series of ω-diphenylaminoalkyl derivatives, ω-diphenylaminopentyl *p*-aminosalicylate exhibited a maximum of $0.0003\times10^{-5}M$/L. that is hundred times that of the parent compound.

The evidences thus obtained support the suggestion that tuberculostatic activities of these derivatives are considerably influenced by both the length of the alkyl chain and the nature of substituents on the end of the alkyl chain. Although no apparent increasing or decreasing in the antitubercular activity by the introductions of chlorine atom and amino group and diethylamine and another PAS moiety can be seen from these data, introductions of phenylethylamine and diphenylamine on the end of the alkyl chain strongly increase the activity of the substance.

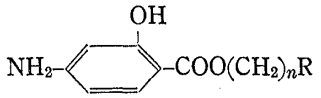
Contrary to the expectation and after the repeated examination, characteristic features of association of activities with the length of alkyl chain could not be seen in the series of ω-morpholinoalkyl, ω-pyrrolidinoalkyl and ω-piperidinoalkyl esters. Slight inclinations, however, of increasing of the activities can be detected in the order of morpholino, pyrrolidino and piperidino at the corresponding length of alkyl chain of the derivatives.

The effects of ω-substituents on the lipid solubility are interesting problem to be solved. The partition coefficients of these synthesized derivatives at 37° between heptane

11) K. Kakemi, *et al.*: This Bulletin, **15**, 1819 (1967)

and phosphate buffered solution having pH 7.4 were determined. The results are listed in Table XIII.

TABLE XIII. Partition Coefficients of PAS Esters at 37° between *n*-Heptane and Phosphate Buffer at pH 7.4



R \ n	2	3	4	5	6	7	8	9	10
chloro	3.12	6.91	9.45	13.2	14.8	20.0	26.4	30.2	48.1
bis	31.1	41.2	54.4	89.1	96.3	156	180	210	230
amino	0.62	0.82	1.02	2.31	2.61	4.91	5.64	14.3	17.5
diethylamino	1.31	3.21	6.63	10.0	12.1	11.9	19.2	23.1	28.2
phenylethylamino	6.34	9.41	15.2	17.4	18.3	20.8	26.7	35.6	42.3
diphenylamino	17.6	25.0	29.3	36.2	43.7	45.5	53.1	64.3	70.1
morpholino	1.75	2.79	3.17	3.92	4.47	4.74	7.33	9.40	11.4
pyrrolidinyl	2.12	4.09	6.53	7.82	11.4	12.7	14.3	21.4	24.5
piperidino	4.08	6.08	7.95	12.2	15.8	21.1	23.0	23.5	28.5
PAS	0.09								

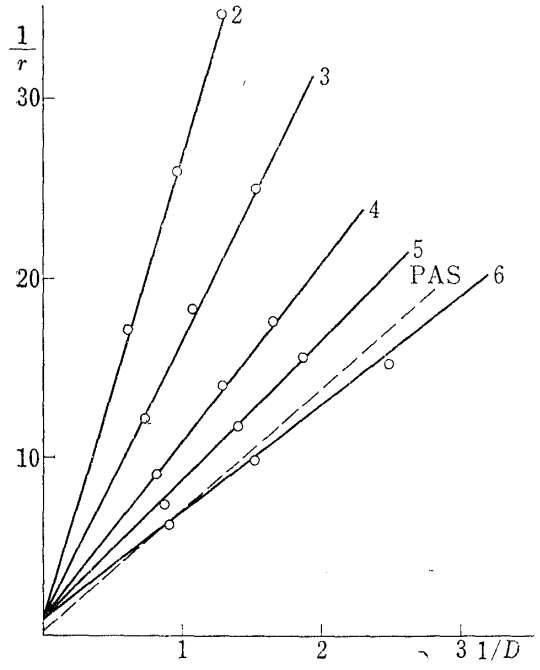


Fig. 1. $1/r-1/D$ Curves of ω-Chloroalkyl *p*-Aminosalicylates

Numbers on the end of each line in Fig. 1 to 9 mean the number of carbon atoms of alkyl chain.

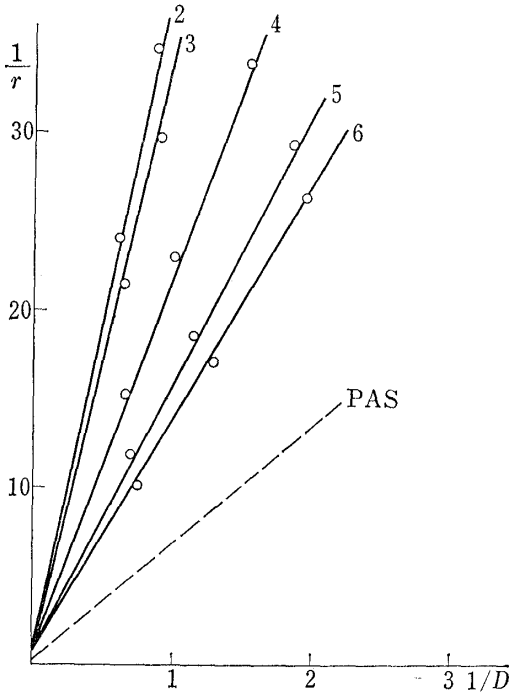


Fig. 2. $1/r-1/D$ Curves of ω-Diethylamino-alkyl *p*-Aminosalicylates

Since it is apparent from the data that all of the newly synthesized compounds have higher lipid solubility than that of the parent compound, these derivatives are suitably designed for transportation in biophase through tissue membranes and easily reach the locus of action.

In connection with the relationship between structure of these derivatives and lipid solubility, the most reasonable conclusions to be drawn from the available data are that

the latter increases with increasing of length of alkyl chain in each homologous series, and that substituents on the end of the alkyl chain are considerably effective upon the lipid solubility. Introduction of another PAS moiety on the end of the alkyl chain brings the coefficient's increasing, on the other hand, amino group considerably decreases the values and chlorine atom has little effect on partition coefficients in comparison with that of alkyl esters, reported in the previous paper.[11]

In comparing the values of ω-aminoalkyl and ω-substituted aminoalkyl esters, it seems to be able to conclude that the displacement of hydrogen atoms of amino group to alkyl or phenyl group results increasing of lipid solubility, and that on influence can be observed by ring closed characteristics on comparing pyrrolidino and diethylamino substituted esters, and that replacing oxygen atom by methylene group brings extensive increasing the coefficients, and that introduction of methylene group in the substituent brings increasing in lipid solubility.

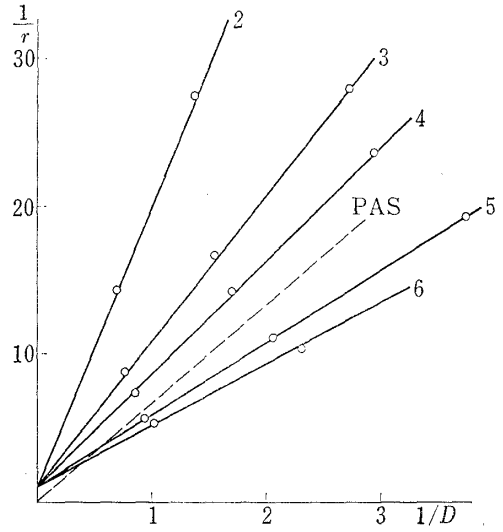


Fig. 3. $1/r-1/D$ Curves of ω-Phenylethylaminoalkyl p-Aminosalicylates

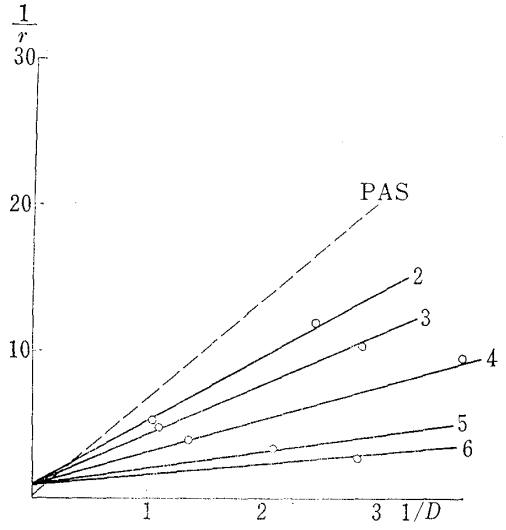


Fig. 4. $1/r-1/D$ Curves of ω-Diphenylaminoalkyl p-Aminosalicylates

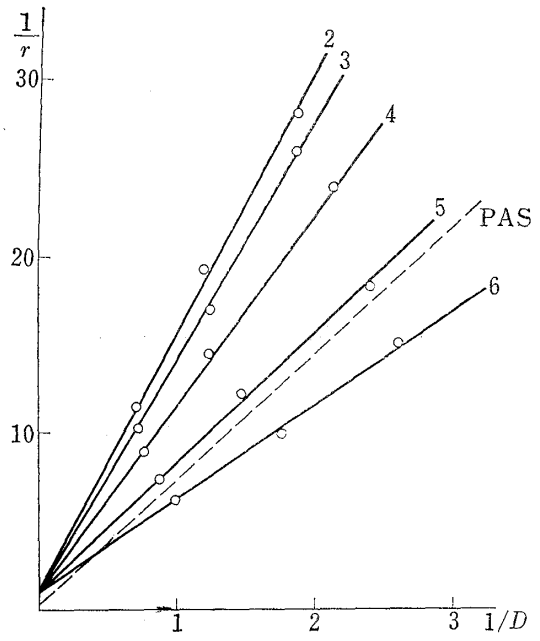


Fig. 5. $1/r-1/D$ Curves of ω-Morpholinoalkyl p-Aminosalicylates

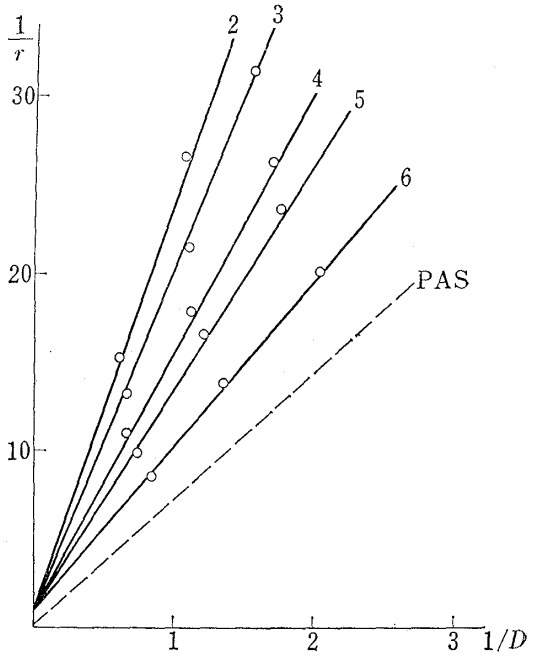


Fig. 6. $1/r-1/D$ Curves of ω-Piperidinoalkyl p-Aminosalicylates

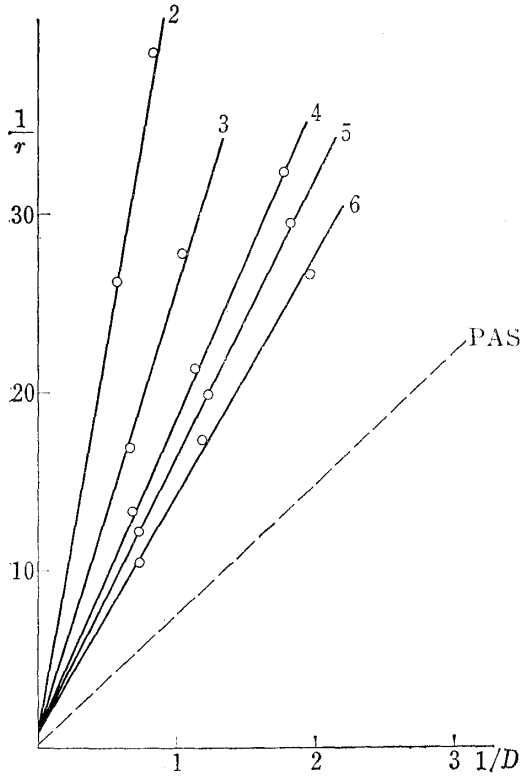


Fig. 7. $1/r-1/D$ Curves of ω-Pyrrolidinyl-alkyl *p*-Aminosalicylates

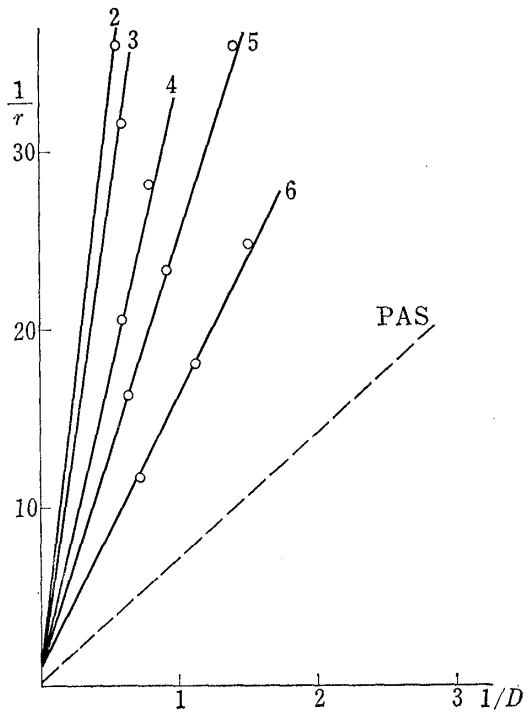


Fig. 8. $1/r-1/D$ Curves of ω-Aminoalkyl *p*-Aminosalicylates

Binding of these derivatives with bovine serum albumin were determined, using the equilibrium dialysis method in phosphate buffered solution having pH 7.4 and ionic strength of 0.05.

The ratio of moles of total albumin to moles of bound compounds ($1/r$) were plotted against a reciprocal of the molar concentration of unbound compound ($1/D$), as shown in Figs. 1 to 9.

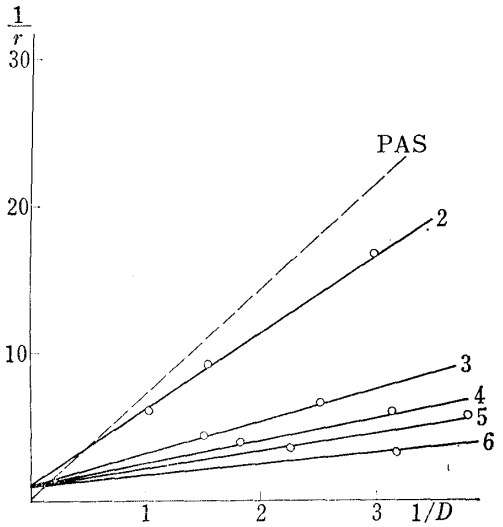


Fig. 9. $1/r-1/D$ Curves of Alkylene bis *p*-Aminosalicylates

As these plots made good linearity, the mode of the interaction of these derivatives to serum albumin were similar to the derivatives reported in the previous paper.[11] Calculated values of n, numbers of binding sites on the albumin molecule, of these derivatives are equally united to the range of 1 ± 0.16 and of the parent compound was revealed closely to 5. There seemed to be no relationship between chemical structure of these derivatives and the numbers of binding sites on albumin molecule and all of these derivatives interact with albumin equally in a 1:1 stoichiometric ratio under the condition of the present experiments, while the parent compound do it with 1:5 ratio.

Since the fractional values of n seemed to be an attribution of experimental errors, K, apparent association constant of the derivatives, were calculated and compared between the series of the derivatives as n were regarded as 1. Calculated values of K and per cent bound at the concentration of $2.0\times10^{-5}M$/L. are shown in Table XIV.

From the table, it is apparent that both of the values of K and per cent bound increase in accompany with the increasing of the length of the alkyl chain and this

Table XIV. Protein Binding of PAS Derivatives

Derivatives: Series	Derivatives: C-Number	Binding sites	K ($\times 10^{-5}$)	Binding ratio at $2.0\times10^{-5}M$/L.
chloro	2	1	0.038	16.2
	3	1	0.057	23.1
	4	1	0.103	32.6
	5	1	0.121	38.6
	6	1	0.161	44.2
bis	2	1	0.187	45.1
	3	1	0.452	65.5
	4	1	0.612	73.0
	5	1	1.32	84.3
	6	1	2.51	86.5
amino	2	1	0.015	8.13
	3	1	0.018	9.21
	4	1	0.028	14.29
	5	1	0.039	16.8
	6	1	0.061	24.4
diethylamino	2	1	0.021	10.5
	3	1	0.028	13.2
	4	1	0.042	18.4
	5	1	0.060	24.5
	6	1	0.068	26.8
phenylethylamino	2	1	0.055	21.3
	3	1	0.085	32.1
	4	1	0.11	38.5
	5	1	0.14	43.7
	6	1	0.16	46.8
diphenylamino	2	1	0.15	45.3
	3	1	0.18	49.3
	4	1	0.36	62.5
	5	1	0.77	78.7
	6	1	0.95	79.2
morpholino	2	1	0.057	25.4
	3	1	0.072	26.3
	4	1	0.092	31.4
	5	1	0.13	39.4
	6	1	0.18	45.3
pyrrolidino	2	1	0.023	11.2
	3	1	0.038	16.6
	4	1	0.054	21.6
	5	1	0.061	23.5
	6	1	0.073	27.4
piperidino	2	1	0.045	19.2
	3	1	0.056	21.2
	4	1	0.068	25.2
	5	1	0.076	27.5
	6	1	0.10	32.4
PAS		5	0.40	42.1

suggests that van der Waals' forces caused by the lengthening of alkyl chain might promote the affinities of binding of these derivatives. The tables reveal that there is an intimate relationship between chemical constitutions on the end of the alkyl chain and K values and per cent bound. The compounds which have another PAS moiety on the ω-position bind to albumin extensively. This finding is also supported in the cases of introductions of phenyl group to ω-amino group and phenyl alkyl esters, reported in the previous paper,[11] this may be attributed to π-electron cloud of the benzene ring and to the increasing of the area formed by the spread of the substituents. Considerable

decreasing of K and per cent bound were observed in the cases of introduction of amino group and chlorine atom in comparison with alkyl derivatives, reported in the previous paper, which can be regarded as no substituent on the end of the alkyl chain. Comparing the data, these findings could be withdrawn in relation to ω-substituted aminoalkyl esters, that no consideable influences could be observed by the difference of ring closed and ring opened characteristics on comparing of pyrrolidino and diethylamino column, that introduction of oxygen atom in the ring increases both values, and that increasing of methylene group in the substituent results significant increasing in apparent association constants and per cent bound.

These preceding observations indicate that the newly synthesized derivatives of PAS have the intrinsic antitubercular activities as same extent as that of the parent compound and at the same time are more lipid soluble and some of them have less protein binding ratio, and it is considered that some of the defects of PAS are extensively improved by these chemical modifications. Furthermore the relationship between the chemical constitutions and these characteristics which affect transportations of substances in biophase are also revealed in this paper.

Experimental

General Method of Syntheses of ω-Chloroalkyl *p*-Aminosalicylates in Which Alkyl Chain are Ethyl to Hexyl——To solution or suspension of 0.033 mole of PAS in 1 mole of corresponding alkylene chlorohydrine, was added 0.165 mole of conc. H_2SO_4 dropwise under stirring and cooling on an ice bath. After the addition of H_2SO_4 was completed, ice bath was changed to an oil bath and the temperature was gradually raised to 90° and kept for 15~20 hr., corresponding to the length of the alkyl chain. The reaction mixture was then concentrated to one third of the initial volume *in vacuo* and precipitates appeared when 10% $KHCO_3$ solution was added to neutralize the mixture. Filtered and purified by recrystallization from appropriate solvents. Melting points, appearances and yields of these compounds are listed in Table I.

General Method of Syntheses of ω-Chloroalkyl *p*-Aminosalicylates in Which Alkyl Chain are Heptyl to Decyl——To mixture of 0.027 mole of PNS and 0.1 mole of corresponding alkylene chlorohydrine, 0.135 mole of conc. H_2SO_4 was added dropwise under stirring and ice cooling. After the addition of H_2SO_4 was completed, ice bath changed to oil bath and then the temperature was gradually raised to 110° and kept for 8~10 hr. After the completion of the reaction was checked by thin-layer chromatography (TLC), the mixture was concentrated to one fourth of the initial volume *in vacuo* and poured into 50 ml. of H_2O, and neutralized with 10% $KHCO_3$ solution. The mixture was extracted with two portions of 50 ml. of ether. The extract was washed with 100 ml. of H_2O and then dried over Na_2SO_4. On removing ether, red brownish viscous oily substances were obtained. The residue was used to next reaction without further purifications.

To a boiling solution prepared from 20 g. of $SnCl_2$ in 55 ml. of EtOH and 20 ml. of conc. HCl, the nitro compound, obtained above, was added gradually. The reaction mixture was refluxed for 5~10 min., and then 250 ml. of H_2O (preliminaly heated about 50°) was added. After cooling to room temperature the, reaction mixture was neutralized with $KHCO_3$ and allowed to stand overnight. The separated oily substances were extracted with benzene and recrystallized from appropriate solvents. Melting points, yields and appearances of these compounds are listed in Table I.

General Method of Syntheses of ω-Diethylaminoalkyl *p*-Aminosalicylates——To stirring and refluxing solution of 0.05 mole of ω-chloroalkyl *p*-aminosalicylate and 0.01 mole NaI in 100 ml. of EtOH, the solution of 0.1 mole of Et_2NH in 50 ml. of EtOH was added dropwisely. The addition required 10~30 min. The mixture was stirred and refluxed for an additional 8 hr. After cooled and filtered off the unreacted ω-chloroalkyl *p*-aminosalicylate and inorganic salt, the filtrate was evaporated to almost dryness *in vacuo*. The residual paste was triturated with 100 ml. of ether and washed with two portions of 50 ml. of 10% $KHCO_3$ solution and then H_2O, and dried over Na_2SO_4. This solution was hydrogenated over 0.5 g. of Pd-C (prepared from 5% $PdCl_2$) at room temperature. After absorption of H_2 was ceased, the filtrates were concentrated *in vacuo*. The residual paste was triturated with 100 ml. of H_2O and then adjusted the pH to 10. Separated precipitates or oily substances were filtered or extracted by solvents and purified by recrystallizations or distillations. The obtained derivatives are listed in Table II.

General Method of Syntheses of ω-Phenylethylaminoalkyl *p*-Aminosalicylates——To a solution of 0.01 mole of phenylethylamine in 100 ml. of toluene was added dropwise a solution of 0.013 mole of $NaNH_2$ in 50 ml. of toluene and then gently warmed. After ceasing the evolution of NH_3, the solvent was distilled until the volume of the mixture was one third of the initial amount. To this concentrated mixture, the solution of 0.01 mole of ω-chloroalkyl *p*-aminosalicylate in 50 ml. of toluene was added dropwise, and after

the addition, the mixture was gently boiled for 3 hr. The reaction mixture was cooled and then filtered. The filtrate was washed with two portions of 50 ml. of 10% $KHCO_3$ solution and H_2O, and dried over Na_2SO_4. The catalytic hydrogenation was proceeded by the method mentioned in the syntheses of *ω*-diethylaminoalkyl *p*-aminosalicylates.

The residual paste was triturated with 100 ml. of H_2O and adjusted the pH to 10. The separated precipitates were filtered and purified by recrystallizations from appropriate solvents. The obtained derivatives are listed in Table Ⅲ.

General Method of Syntheses of *ω*-Diphenylaminoalkyl *p*-Aminosalicylates——To warmed solution of 0.01 mole of diphenylamine in 100 ml. of toluene, was added dropwise a solution of 0.015 mole of $NaNH_2$ in 50 ml. of toluene with cautions and warming continued for further several minutes. After ceasing the evolution of NH_3, the solvent was distilled until the volume of the mixture was one third of the initial amount. To this concentrated mixture, the solution of 0.01 mole of *ω*-chloroalkyl *p*-nitrosalicylate in 50 ml. of toluene was added dropwisely, and the mixture was gently boiled for 3～4 hr. Then the mixture was cooled and filtered, the filtrate was washed with two portions of 50 ml. of 10% $KHCO_3$ and then with H_2O, and dried over Na_2SO_4. The catalytic hydrogenation of the filtrate was proceeded by the method mentioned in the syntheses of *ω*-diethylaminoalkyl *p*-aminosalicylates.

The residual paste was triturated with 100 ml. of H_2O and adjusted the pH to 10. The separated precipitates were filtered and purified by recrystallizations from appropriate solvents. The obtained derivatives are listed in Table Ⅳ.

General Method of Syntheses of *ω*-Morpholino-, *ω*-Pyrrolidinyl- and *ω*-Piperidino-alkyl Alcohols——A mixture of 1 mole of alkylene chlorohydrine, 2 mole of morpholine (or pyrrolidine, or piperidine) and 0.052 mole of NaI were dissolved in 200 ml. of EtOH and the solution was refluxed for 24 hr., with stirring. Precipitates appeared during the course of the reaction were filtered off, the filtrate was concentrated to almost dryness. The residual paste was purified by distillation *in vacuo*.

General Method of Syntheses of *ω*-Morpholinoalkyl, *ω*-Pyrrolidinylalkyl and *ω*-Piperidinoalkyl *p*-Aminosalicylates——To stirred and ice cooled 0.3 mole of *ω*-morpholinoalkyl alcohols, the equimoles of conc. H_2SO_4 was added with cautions. One tenth mole of PAS was added to sticky and yellow colored sulfate of *ω*-morpholinoalkyl alcohols, and then 0.4 mole of conc. H_2SO_4 was added dropwisely and then the ice bath was changed to oil bath. The reaction temperature was kept not more than 90°, and the mixture stirred for 15～24 hr. corresponding to the length of the alkyl chain. When the reactions were completed, the resulted mixture was poured onto ice water. After adjusting the pH of the solution to 10 by adding $NaHCO_3$, the separated precipitates or oily substances were filtered or extracted by appropriate solvent and purified recrystallizations or distillations. The obtained derivatives are listed in Table Ⅴ, Ⅵ, Ⅶ.

General Method of Syntheses of *ω*-Aminoalkyl *p*-Aminosalicylates——Under vigorous stirring and cooling by dry ice and acetone to 30°, 1 mole of conc. H_2SO_4 was added dropwisely to 1 mole of *ω*-aminoalkyl alcohol. To this mixture 0.5 mole of PNS and 2 mole of conc. H_2SO_4 were added portionwisely and the temperature of the mixture was maintained at 110° for 8～15 hr., corresponding to the length of the alkyl chain. After completion of the reaction was checked by thin-layer chromatography, the mixture was poured into a small amount of water and then pH of the solution was adjusted to 10 by adding KOH. Separated oily substances were extracted by two portions of 100 ml. of EtOAc, washed with H_2O and then dried over Na_2SO_4. Oily substances obtained on removing the organic solvent, was reduced catalytically without further purifications. The method of the reduction of these substances were followed by the method mentioned above. The resulted hydrogenation product was submitted to chromatography over Kieselgel G with benzene and recrystallized from appropriate solvents. The obtained substances are listed in Table Ⅷ.

General Method of Syntheses of Alkylene bis-*p*-Aminosalicylates——To a solution of 2.5 mole of corresponding alkylene glycol in 100 ml. of ether, 1 mole of nitrosalicyloyl chloride was added gradually, under vigorous stirring. After addition of the chloride, the mixture was gently boiled for 4～8 hr. Being checked by TLC of the completion of the reaction, the mixture was washed 5 portions of 20 ml. of 10% $KHCO_3$ and then the organic layer was dried over Na_2SO_4. The residual yellowish viscous liquid was obtained on removing the solvent, and was hydrogenated catalyticaly without further purifications (see above). The resulted pale yellow crystals were dried over P_2O_5 recrystallized from appropriate solvents. The obtained substances are listed in Table Ⅸ. General method of determination of antitubercular activities, partition coefficients and protein binding characteristics of the compounds were described in the previous report.

Authors express their gratitude to Prof. M. Naito and Dr. S. Tsukuma of Tuberculosis Research Institute of Kyoto University for their kind supply to the test of antitubercular activities of the synthesized compounds and to Dr. K. Konobu, director of Elemental Analysis Center of Kyoto University, for elemental Analyses of the substances.