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232. Kiichiro Kakemi, Takaichi Arita, Shikifumi Kitazawa, Mitsuo Kawamura, and Hiroshi Takenaka*1 : Studies on the Pharmaceutical Potentiation of Drugs. I. *p*-Aminosalicylic Acid Derivatives.

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Three series of PAS derivatives, alkyl, ω-hydroxyalkyl, and ω-phenylalkyl esters, were synthesized systematically and their evaluations were made with testing their tuberculostatic activities and with measuring their physico-chemical characteristics, such as partition coefficients and protein binding ratio that might considerably influence biological effects in animal body.

It is recognized that these derivatives may extensively improve the defects of the parent compound. It is also demonstrated that the substituents on the end of the alkyl chain and the length of the alkyl chain of these compounds considerably influence on their antitubercular activities and physico-chemical properties.

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It is well known that the therapeutic efficacy of a compound is not only a function of its effect at the locus of action but also its ability to reach and furthermore, stay there to exert its therapeutic effect.

It is now recognized that a drug with a high activity *in vitro* might exert no biological effects *in vivo*, because its physico-chemical characteristics do not permit it to reach the particular site of action. Therefore, a knowledge, combined with a traditional evaluation of intrinsic activity by screening test, of physico-chemical properties of a compound which influence its transportation in biophase, such as absorption, protein binding, distribution in tissues, enzymatic and non-enzymatic biotransformation, excretion, and so on, should be essential for a complete evaluation of the compound.

From these points of view, potentiation of drugs in animal body can also be achieved by some modification of their chemical constitutions to be readily transported to the site of action.

para-Aminosalicylic acid (PAS) was found to have high tuberculostatic activity *in vitro* for virulent human type tubercule bacilli,[1] but when used clinically, relative large dosage, 15~20 g. daily, is necessary.[2] It is not palatable and causes many undesirable side effects. This large dosage may be due to the fact that PAS is rapidly excreted,[3]

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1) J. Lehman : Lancet, **250**, 15 (1946).
2) K. Alin, H. Difs : Nord. Med., **33**, 151 (1947).
3) E.L. Way, *et al.* : J. Pharmacol. Exptl. Therap., **111**, 197 (1954).

highly protein bound,[4] competitively antagonized with structurely similar *p*-aminobenzoic acid (PABA),[5] susceptible to biotransformation which yields a number of inactive metabolites,[6] and after all, effective amount of the compound can not reach to the locus of action.

In general, individual tissue cell has a boundary characterized by lipoid membrane, and moreover, *Myc. tuberculosis* exists in the lipoid tissue and they have thick lipoid membrane.[7]

Lipid soluble substances can readily penetrate tissue cells and be transported to sites of action, so that the lipid solubility, that is, oil-water partition coefficients of the compound could be one of the indices of the permeability or transportation of the compound to the locus of action in biophase.[8,9]

On the other hand, in plasma, organic substances bind with plasma protein, mainly with serum albumin. Since protein bonnd substances, forming macromolecule, can not diffuse and penetrate through membranes, protein binding ratios of substances *in vitro* may also be one of the indices of transportation or distribution of the substances in biological fluid.[10,11]

Potentiation of PAS is able to be achieved by chemical modifications of the parent compound to increase their oil-water partition coefficients and to decrease their protein binding ratios, so that it will be possible to diminish the dosage, even if with the compounds that could not have the same tuberculostatic activities as the parent compound.

For these purposes, derivatives of PAS were synthesized with rational molecular design and made their practical evaluations with such data as their intrinsic activities at the locus of action and their aptitude of transportation to the locus. To the former, investigations were made by the traditional screening test using human uirulent tubercule bacilli H37Rv strain, and to the latter, their partition coefficients between heptane and buffered solution and binding ratios to serum albumin were measured *in vitro* under conditions designed similarily as that might occur in biophase as possible.

Since days of Lehman's discovery, many reports have been published on the syntheses of PAS derivatives with evaluating only by their *in vitro* screening test.[12~14] These reports suggested that derivatives with substituents on the *ortho*-hydroxy and *para*-amino groups decrease their activities,[15,16] and, furthermore, that had substituents on 3, 5 and 6 positions of the benzene ring lost their antitubercular activities.[17] Derivatives, however, of carboxylic acid group, such as esters have the activities to some extent.

Carboxylic acid esters of PAS were synthesized in this paper, especially, alkyl, ω-hydroxyalkyl and ω-phenylalkyl esters with their alkyl chain systematically from methyl to octadecyl in the case of alkyl derivatives, ethyl to decyl in the case of ω-hydroxyalkyl derivatives and methyl to propyl in the case of ω-phenylalkyl derivatives.

4) J. Gomi : Saishin Igaku, **32**, 1 (1957).
5) G.P. Youman, *et al.* : J. Bacteriol., **54**, 409 (1947). L.W. Hedgecock : *Ibid.*, **75**, 345, 417 (1958).
6) M. Nakao : J. Biochem., **44**, 327, 433, 477 (1957); *Ibid.*, **45**, 757 (1958).
7) B.L. Freedlander : Am. Rev. Tuberc., **56**, 376 (1947).
8) B.B. Brodie, *et al.* : J. Pharmacol. Exptl. Therap., **123**, 81 (1958).
9) B.B.Brodie, *et al.* : J. Pharm. Pharmacol., **9**, 345 (1957).
10) I.M. Klotz : "Protein Structure and Function," Brookhaven Symposia in Biology, No. **13**, 25 (1960).
11) A. Goldstein : Pharmacol. Rev., **1**, 102 (1949).
12) A. Wander : Swiss Patent 265667.
13) W. Grimme : German Patent 842944; Chem. Ber., **84**, 734 (1951).
14) Hans V. Euler : Ark. Kemi., **2**, 297 (1950).
15) R. Hirt, H. Hunri : Helv. Chem. Acta, **32**, 378 (1949).
16) D.J. Drain, *et al.* : J. Pharm. Pharmacol., **1**, 784 (1949).
17) H. Bretschneider, W. Kloetzer : Monat., **81**, 781 (1950).

Of these derivatives, methyl to pentyl in the homologous series of alkyl esters, ethyl of ω-hydroxyalkyl esters and methyl of ω-phenylalkyl esters are known in literatures.

In the studies of preparation of methyl, ethyl and propyl esters of PAS, previously reported by Drain[18,19] and others, were prepared by direct esterification of PAS with corresponding alcohols under refluxing in the presence of conc. sulfuric acid and butyl and pentyl esters, however, direct esterifications yielded only *meta*-aminophenol, a decarboxylated product of the material, owing to high boiling points of alcohols and decarboxylation occurred during the course of this reaction.

Drain synthesized these esters by using ω-nitrosalicylic acid (PNS) and the alcohols in the presence of conc. sulfuric acid under heating to obtain alkyl nitrosalicylates and then reduced to amino derivatives. A modification of Drain's method that is applicable for syntheses of ester which have longer alkyl chain, was considered with using PAS in the place of PNS. Since, although PAS is obtainable commercially, PNS must be synthesized by the procedures followed that of Bhate,[20] and the yield of the compound is not so good.

Considerable experimental works were carried out in the determination of a suitable procedure for the preparation of the longer chain alkyl esters. Direct esterification of PAS by means of alkyl alcohols and conc. sulfuric acid at the temperature of more than 95° for eight hours gave *meta*-aminophenol. On the other hand at 85° gave the material unchanged. Because of the results obtained in the above procedure, attention was attracted to the temperature of the reaction.

Successful direct esterification method could be found at the temperature of 90°, and the reaction time of 15~24 hr. corresponding to the length of alkyl chain, in which the decarboxylation could be suppressed at least extent during the course of the reaction. The esters thus obtained are listed in Table I.

In the studies of preparation of ω-hydroxyalkyl *p*-aminosalicylates, previously reported by Wander,[12] was prepared by the direct esterification of PAS by means of ethylene glycol and conc. sulfuric acid. The reaction mixture obtained from the above procedure contained much resinous substances and seemed to be difficult for further purifications. Since the procedure of Wander had proved consistently successful, it was thought advisable to investigate another procedure for the preparation of this homologous series of the derivatives with higher length of alkyl chain.

The direct esterification of PNS with alkylene glycols and conc. sulfuric acid also gave poor yields, and the product were quite difficult to purify.

Because of the difficulties encountered in the above procedure, attention was turned to the utilization of nitrosalicyloyl chloride, and this route offered a possible source of the desired products and purifications were fairly proceeded.

The reactions of nitrosalicyloyl chloride with alkylene glycol were proceeded in ether under refluxing. To avoid the formations of diester derivatives, the heating time was limited for only one hour and then allowed to stand overnight. Since the nitro compounds thus obtained were somewhat unstable, the reduction to the corresponding amino compounds were proceeded immediately without further purifications.

Nitrosalicyloyl chloride, used in the above syntheses, were prepared according to the directions of Otto Clauder.[21]

The esters thus obtained are listed in Table II.

On the preparations of homologous series of ω-phenylalkyl *p*-aminosalicylates, the direct esterification of PAS by means of phenylalkyl alcohols and conc. sulfuric acid

18) D. J. Drain, D. D. Martin : J. Chem. Soc., **1952**, 3861.
19) W. O. Godfredsen, *et al.* : Acta Chem. Scand., **7**, 781 (1953).
20) D. S. Bhate, *et al.* : Proc. Indian Acad. Soc., **29** A, 196 (1949).
21) O. Clauder, *et al.* : Magyar Kem. Lapya., **4**, 596 (1949) (Chem. Abst., **47**, 115 (1953)).

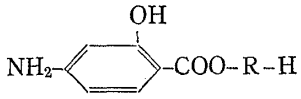
gave poor results. *p*-Nitrosalicyloyl chloride was reacted with phenylalkyl alcohols at room temperature without any solvent and then reduced to the corresponding amino compounds by stannous chloride and hydrochloric acid procedure. The major products were proved to be the desired substances.

The products thus obtained are listed in Table Ⅲ.

The *in vitro* determinations of the tuberculostatic activities of these PAS derivatives were carried out by measuring the minimum inhibitory concentrations against the virulent H37Rv strain of *Myc. tuberculosis*. The results are summarized in Table Ⅳ.

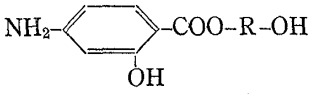
One of the most notable features is that the intrinsic activities are clearly associated with the alkyl length of each homologous series of these derivatives. Compounds with very short alkyl length exhibit little of action, but as the chain are lengthened, the

Table I. Alkyl *p*-Aminosalicylates



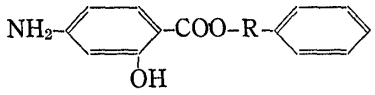
R	Appearances	m.p. (°C)	Recrystal. solvent	Yield (%)	Formula	Analyses (%) Calcd. C	H	N	Found C	H	N
CH_2	colorless needles	121	EtOH	76.0	$C_8H_9O_3N$	57.48	5.48	8.38	57.42	5.32	8.21
C_2H_4	colorless needles	111	EtOH	80.0	$C_9H_{11}O_3N$	59.66	6.12	7.73	59.38	6.12	7.51
C_3H_6	colorless needles	103	EtOH	78.5	$C_{10}H_{13}O_3N$	61.52	6.71	7.18	61.81	6.53	7.32
C_4H_8	colorless needles	93	EtOH	70.0	$C_{11}H_{15}O_3N$	63.14	7.23	6.69	63.01	7.31	6.39
C_5H_{10}	colorless plates	74	Me_2CO	56.0	$C_{12}H_{17}O_3N$	64.55	7.68	6.27	64.36	7.46	6.51
C_6H_{12}	pale-yellow plates	67.5	EtOH	43.0	$C_{13}H_{19}O_3N$	65.80	8.07	5.90	65.59	8.31	5.69
C_7H_{14}	colorless plates	57	EtOH	46.0	$C_{14}H_{21}O_3N$	66.90	8.42	5.57	66.71	8.53	5.37
C_8H_{16}	pale-yellow needles	64	EtOH	43.0	$C_{15}H_{23}O_3N$	67.89	8.74	5.28	67.59	8.82	5.31
C_9H_{18}	colorless powder	59	EtOH	46.0	$C_{16}H_{25}O_3N$	68.78	9.02	5.01	68.91	9.23	5.21
$C_{10}H_{20}$	colorless plates	65.5	benzene	26.0	$C_{17}H_{27}O_3N$	69.59	9.28	4.77	69.32	9.51	4.71
$C_{12}H_{24}$	colorless plates	68	benzene	48.0	$C_{19}H_{31}O_3N$	70.99	9.72	4.36	70.72	9.85	4.56
$C_{14}H_{28}$	colorless plates	73	benzene	40.0	$C_{21}H_{35}O_3N$	72.16	10.09	4.01	72.21	10.41	4.40
$C_{16}H_{32}$	colorless needles	86	benzene	40.9	$C_{23}H_{39}O_3N$	73.16	10.41	3.71	73.27	10.48	3.54
$C_{18}H_{36}$	colorless plates	92	benzene	41.1	$C_{25}H_{43}O_3N$	74.03	10.69	3.45	74.21	10.39	3.51

Table II. ω-Hydroxyalkyl *p*-Alminosalicylates



R	Appearances	m.p. (°C)	Recrystal. solvent	Yield (%)	Formula	Analyses (%) Calcd. C	H	N	Found C	H	N
C_2H_4	colorless needles	134	Me_2CO+MeOH	30.0	$C_9H_{11}O_4N$	54.82	5.62	7.10	54.70	5.53	6.99
C_3H_6	colorless needles	83	Me_2CO+MeOH	42.0	$C_{10}H_{12}O_4N$	56.86	6.20	6.63	56.63	6.22	6.65
C_4H_8	colorless prisms	69	Me_2CO+MeOH	41.5	$C_{11}H_{15}O_4N$	58.65	6.71	6.22	58.88	6.41	6.61
C_5H_{10}	colorless needles	58	Me_2CO	54.6	$C_{12}H_{17}O_4N$	60.24	7.16	5.85	59.95	7.36	5.70
C_6H_{12}	colorless needles	42	Me_2CO	40.0	$C_{13}H_{19}O_4N$	61.64	7.56	5.53	61.48	7.39	5.52
C_7H_{14}	colorless prisms	68	Me_2CO	33.0	$C_{14}H_{21}O_4N$	62.90	7.92	5.24	62.78	8.03	4.99
C_8H_{16}	colorless plates	110	Me_2CO+H_2O	22.6	$C_{15}H_{23}O_4N$	64.03	8.24	4.98	63.98	7.99	4.98
C_9H_{18}	colorless needles	115	Me_2CO+H_2O	30.0	$C_{16}H_{25}O_4N$	65.06	8.53	4.53	65.13	8.49	4.73
$C_{10}H_{20}$	colorless needles	120	Me_2CO+H_2O	20.0	$C_{17}H_{27}O_4N$	65.99	8.80	4.53	65.78	8.98	4.41

Table Ⅲ. ω-Phenylalkyl *p*-Aminosalicylates



R	Appearances	m.p. (℃)	Recrystal. solvent	Yield (%)	Formula	Analyses (%) Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
CH_2	colorless plates	99	ligroin	66.4	$C_{14}H_{13}O_3N$	69.12	5.39	5.76	69.41	5.09	5.72
C_2H_4	colorless plates	71.2	ligroin	59.3	$C_{15}H_{15}O_3N$	70.02	5.88	5.44	70.18	5.59	5.55
C_3H_6	colorless plates	84.5	ligroin	55.6	$C_{16}H_{17}O_3N$	70.83	6.32	5.16	70.72	6.36	5.23

Table Ⅳ. Minimum Inhibitory Concentrations of the Derivatives on the Growth of *Myc. tuberculosis* H37Rv in Kirchner's medium ($\times 10^{-6}M$/L.)

C-Number	ω-Substituents Alkyl	Hydroxyalkyl	Phenylalkyl
1	14		1.6
2	14	12.6	3.2
3	6.4	2.3	0.2
4	2.9	0.55	
5	1.4	0.52	
6	0.54	0.25	
7	0.49	0.015	
8	0.47	0.11	
9	0.44	0.42	
10	0.85	0.81	
12	0.77		
14	1.4		
16	2.6		
18	2.4		
PAS	0.25		

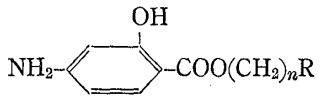
activities increased, reaching a maximum, and further lengthening bring decrease in the activities. Same inclinations were observed by Aoyagi[22] and Watanabe[23] in the cases of series of fatty acid and aminoalkoxybenzene homologous. In both cases the peak of tuberculostatic activities were exhibited at carbon number of the alkyl chain was twelve. But in the cases of these homologous series of alkyl esters of PAS, nonyl *p*-aminosalicylate had a maximum activity of $0.44\times10^{-5}M$/L., that is as same as that of PAS, in the series of alkyl esters, and in ω-hydroxyalkyl series, ω-hydroxyheptyl *p*-aminosalicylate exhibited a maximum of $0.015\times10^{-5}M$/L., that is superior than that of the parent compound.

The evidences thus obtained support the suggestion that tuberculostatic activities of these derivatives are wholy influenced by both of the length of alkyl chain and substituents on the end of the alkyl chain. Introduction of hydroxy group on the end of the alkyl chain would have the inclination of activating the action as can be seen from the table.

22) T. Aoyagi : This Bulletin, **5**, 218, 224 (1957).
23) H. Watanabe : Yakugaku Zasshi, **71**, 713 (1951); **72**, 543 (1952); **74**, 872 (1954).

In an attempt to determine the lipid solubility resulted by these chemical modifications and to presume the adaptabilities of their transportation to the locus of action, the partition coefficients of these newly synthesized compounds at 37° between heptane and phosphate buffered solution having pH 7.4, similar pH with that of body fluids, were determined. The results are listed in Table V.

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



n	R		
	H	Hydroxyl	Phenyl
1	1.92		33.1
2	9.24	0.15	103
3	10.33	0.21	154
4	14.7	1.50	
5	21.1	1.90	
6	25.3	3.20	
7	26.1	7.31	
8	29.9	10.3	
9	56.9	14.2	
10	121	17.3	
PAS	0.09		

Since it is apparent that all of the compounds have higher lipid solubilities than that of the parent compound, these compounds are profitable for transportation in biophase through tissue membranes and easily reach to the locus of action.

Lipid solubility increases with increasing of alkyl chain length in each homologous series. The effect of the substituent at the end of the alkyl chain is considerable toward the partition coefficients. Hydroxyl group considerably decreases the coefficients and, on the other hand, phenyl group increases the values, in comparison with alkyl esters. Phenyl esters have the highest coefficients and more than the propyl esters, the values seemed to be infinite, this is the reason for ceasing the synthesis of this series at the propyl ester.

Binding of these derivatives with bovine serum albumin was determined using the equilibrium dialysis method in phosphate buffered solution having pH 7.4 and ionnic 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 compounds ($1/D$), as shown in Fig. 1 and 2. As these plots made good linearity, the mode of the interaction could be fitted to Klotz's equation.[24]

$$1/r = 1/nK \times 1/D + 1/n$$

where r is ratio of moles of bound compounds to moles of protein, K is apparent association constant, D is moles of unbound compound, and n is numbers of binding site on the protein molecule.

24) I.M. Klotz: J. Am. Chem. Soc., **68**, 2299 (1946).

As the equation requires, the intercepts of these extrapolated lines on the ordinate represent $1/n$. The reciprocal of $1/n$, is almost equally to 1 in these derivatives, while n of PAS is about 5. Values obtained from the calculations, n of these derivatives are equally united to 1 ± 0.15. It seems likely that there is no relationship between chemical structure of these derivatives and number of binding site on albumin molecule. All of these derivatives interact with albumin in a 1:1 stoichiometric ratio under the conditions of the present experiments.

Since the fractional value of n must be attributed to experimental error, K were calculated between the series as n was 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 Ⅵ.

Table Ⅵ. Protein Binding of PAS Derivatives

Derivatives		Binding sites	K ($\times10^{-5}$)	Binding ratio at $2.0\times10^{-5}M$/L.
Series	C-Number			
Alkyl	1	1	0.041	15.3
	2	1	0.062	21.3
	3	1	0.19	43.3
	4	1	0.25	47.0
	5	1	0.65	71.3
Hydroxyl	2	1	0.015	6.0
	3	1	0.016	7.21
	4	1	0.040	13.2
	5	1	0.045	13.3
Phenyl	1	1	1.38	84.4
	2	1	1.15	81.8
	3	1	1.07	79.3
PAS		5	0.404	42.1

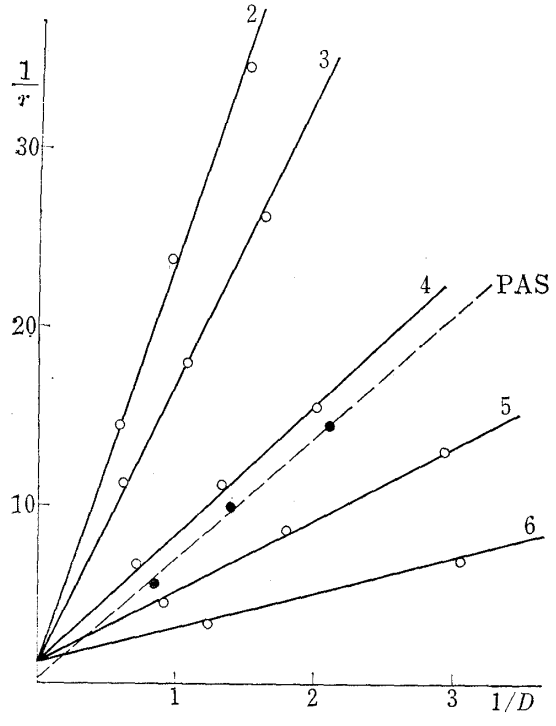


Fig. 1. $1/r-1/D$ Curves of Alkyl *p*-Aminosalicylates

Numbers on the end of each line in Fig. 1 and 2 mean the number of carbon atoms of alkyl chain.

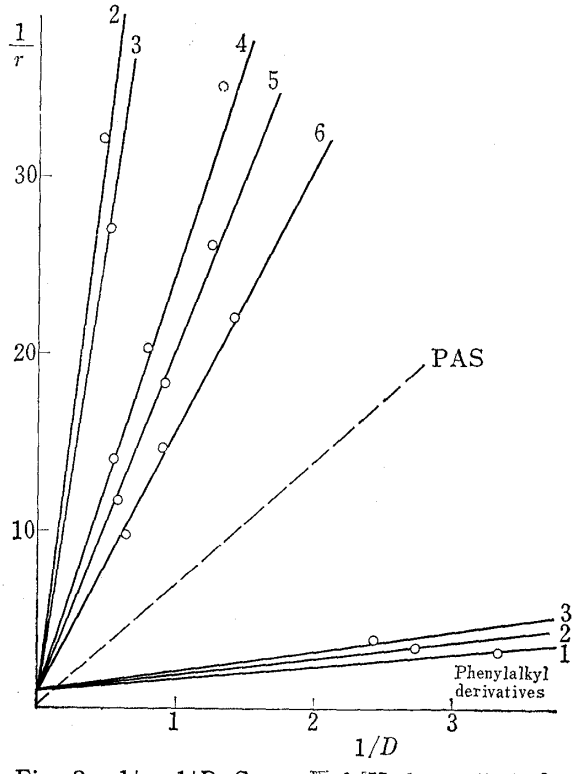


Fig. 2. $1/r-1/D$ Curves of Hydroxyalkyl & Phenylalkyl *p*-Aminosalicylates

From the Table, it seemed that there is intimate relationship between substituents on the end of the alkyl chain and K values.

In alkyl and hydroxyalkyl series, the increasing effect of the degree of protein binding with lengthening of the alkyl chain, suggests that, as Goodman[25] and Teresi[26] pointed out, van der Waals' forces promote the affinities of binding. Very interesting comparison will be made between three kinds of substituents on the end of the alkyl chain. Phenyl radical caused more extensive binding than alkyl and hydroxy radicals. This suggests that π-electron cloud on phenyl nuclei promotes the binding affinities. Since the association constants of these phenyl derivatives are approximately similar, this effect is greater than the effect caused from van der Waals' forces by the lengthening of the alkyl chain. On the other hand, however, the hydroxy radical on the alkyl ends decreases the binding affinities, it is interesting that Davison and Smith[27] reported that hydroxy radical on aromatic ring enhanced binding affinities of benzoic acid and three isomers of hydroxybenzoic acid to albumin. But from the data obtained here, it is apparent that hydroxy radical on the end of the alkyl chain decrease the affinities, comparing to that of alkyl compounds.

The evidences thus obtained support the suggestion that these newly synthesized derivatives are more lipid soluble and some of them are less protein bound in plasma than that of the parent compound and it is considered that the defects of PAS are extensively improved by these chemical modifications. Lipid solubility increase in order of hydroxy, alkyl and phenyl, while protein binbing affinities decrease in order of phenyl, alkyl and hydroxy substituents on the end of the alkyl chain.

Experimental

General Method of Syntheses of Alkyl *p*-Aminosalicylates——To the solution or suspension of 0.033 mole of PAS in 1 mole corresponding *n*-alkylalcohols, 0.165 mole of conc. H_2SO_4 was added dropwisely under stirring and cooling in ice bath. After the addition of H_2SO_4, the bath was changed to oil bath and the temperature of the reaction was gradually raised to 90° and kept the temperature for 15~24 hr. during the esterification. The reaction mixture was concentrated to one third of the volume *in vacuo*, and precipitates appeared, when 10% Na_2CO_2 solution was added to neutralize the concentrated mixture. Filtered and purified by recrystallizations from appropriate solvents. Melting points, yields, analyses and appearances of these esters are listed in Table I.

General Method of Syntheses of ω-Hydroxyalkyl *p*-Aminosalicylates——One and a half mole of nitrosalicyloyl chloride was added to the solution of 1.5 mole of corresponding alkylene glycols in ether. The mixture was refluxed for 1 hr. and yellow crystals appeared after standing over night. The crystals were filtered and dried over P_2O_5 and was used to next reaction without further purifications.

To a boiling solution of 20 g. of $SnCl_2$ in 55 ml. of EtOH and 20 ml. of conc. HCl, 0.025 mole of nitro compound, obtained above, was added gradually. The mixture was refluxed for further 5~10 min. and then 250 ml. of H_2O, preliminary heated to about 50°, was added. After cooling to room temperature the separated amino compounds were filtered and washed with H_2O. Purification was made by the recrystallization from appropriate solvents.

Melting points, yields, analyses and appearances of these compounds are listed in Table II.

General Method of Syntheses of ω-Phenylalkyl *p*-Aminosalicylates——Suspensions were made by adding 1 mole of nitrosalicyloyl chloride to the same mole of corresponding phenylalkyl alcohols. The mixture was stirred at room temperature for 15 min. until the evolution of HCl ceased, and subsequently cooled to room temperature. The crystals which appeared were filtered and dried over P_2O_5 and reduced without further purifications. The method of reduction of these derivatives was similar that of used in the cases of ω-hydroxyalkyl esters. Purification was made by the recrystallizations from appropriate solvents. Melting points, yields, analyses and appearances of these compounds are listed in Table III.

Antitubercular Activity——Antitubercular test of the synthesized compounds were carried out using *Mycobacterium tuberculosis* H37Rv and Kirchner's medium containing 10% of bovine serum.

25) D. S. Goodman: J. Am. Chem. Soc., **80**, 3892 (1958).
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The test compounds were dissolved in the medium, but compounds with poor solubility in water were dissolved in 20% of propylene glycol, and then diluted with the medium by the two fold serial dilution method and then inoculated with the organisms. The medium were incubated at 37° for 4 weeks.

Minimum inhibitory concentrations were determined with observing the growth of tubercule bacilli macroscopically from the outside of the culture tubes after four weeks incubation.

Analytical Measurement——Ultraviolet spectrophotometry was employed throughout the following experiments. The wavelength of the maximum absorption of the homologous series are same; alkyl ester series have at 302 mμ (H_2O), phenylalkyl series at 305 mμ (H_2O+EtOH) and hydroxyalkyl series at 305 mμ (H_2O).

Partition Coefficients——Solution containing three different concentrations of the synthesized aminosalicylates were prepared with pH 7.4 of phosphate buffered solution. Five ml. portions of the solutions were added to the equal volume of heptane. They were kept in a constant temperature water bath at 37° with removal for vigorous shaking for 1 min. every 10 min. interval for 1 hr. Aqueous phase was measured by ultraviolet spectrophotometrically. Partition coefficients were calculated as means of partition ratios of three different concentrations. Partition ratio was calculated by following equation :

$$\text{P.R.} = \frac{\text{(initial conc. of aqueous phase)} - \text{(equlid. conc. of aqueous phase)}}{\text{(equild. conc. of aqueous phase)}}$$

Dialysis Experiments for Measuring Protein Binding——Since there is a general agreement that albumin is uniquely adapted for binding experiments of variety of molecules, crystalline bovine serum albumin (BSA, obtained from Armour Pharmaceutical Company, Kankakee, Illinois, USA) was used in this experiment.

Buffered solutions having pH 7.4 and ionnic strength of 0.05 employed in this experiment were made from reagent grade phosphate salts.

The bags for dialysis were prepared from 8/32 cellophane tubes obtained from Visking Company, Chicago, Illinois, USA.

All tubings were thoroughly washed with running water for one hr., rinsed with distilled water, and soaked in the buffered solution until used.

Cellophane tubings, filled with 4 ml. of 1% solution of BSA, were immersed in 9 ml. of solution of synthesized compounds and placed in a cold room at the temperature approximately 4° for the period of 12 hr. sufficient period for attainment of the equilibrium. For each concentration, a control tube was also prepared which differed from the primary tube only in that the former contained the buffer than a BSA solution inside of the tube. By this method it was possible to minimize any errors arising from binding of the compounds to the cellophane membrane.

After attainment the equilibrium the solution outside of the bag was pippetted out and the concentrations were measured by ultraviolet spectrophotometer to obtain the degree of the binding.

An examination of literature reveals that the Donnan's effect for BSA, when contained 1% of the solution, would be completely negligible,[28] so that the corrections for this effects were not necessary.

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