Discussion and Conclusion

We investigated the possible pharmacological activity of crude drugs that have been used traditionally to treat OKETSU, but failed to find any drugs having diuretic and anti-edematous actions. In contrast, crude drugs containing saponin showed anti-edematous and diuretic actions. Mi-saponin showed anti-edematous and diuretic actions that were dose-dependent, *i.e.*, essentially proportional to the amouht of saponin present in the crude drugs. Therefore, active ingredients were assumed to be present in the saponin. Concomitant use of phenylbutazone with Mi-saponin resulted in an increase in urine volume and anti-edematous action. Further study seems worthwhile. A previous study[9] failed to find anti-inflammatory and anti-edematous actions of crude drugs of Cocculus. However, an alkaloid of Cocculus, trilobine, showed apparent anti-edematous action on congestive edema in this work. Further studies on active ingredients of crude drugs which showed activity in the present experiments are in progress.

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UDC 547.587.11.04.09 : 615.272.4.011.5.015.11

Chemistry of Salicylic Acid and Anthranilic Acid. III.[1] Hypoglycemic Screening Tests for Salicylic and Anthranilic Acid Derivatives

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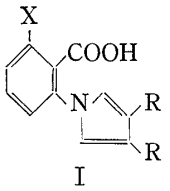
The hypoglycemic activities of O-substituted salicylic acids (**1**—**11**), N-substituted anthranilic acids (**18**—**27**) and related compounds (**12**—**17**, **28**—**30**) were investigated. It was concluded that the presence of a chlorine atom *ortho* to the carboxy group in salicylic and anthranilic acid derivatives might enhance the hypoglycemic activity.

Keywords—— salicylic acid derivatives; thiosalicylic acid derivatives; anthranilic acid derivatives; chlorine atom *ortho* to carboxy group; hypoglycemic activity

Although salicylates have long been known to have hypoglycemic activity, their usefulness as hypoglycemic agents has been limited by the large doses required to bring about a significant lowering of blood glucose.[3,4] However, the effectiveness of sodium salicylate or acetylsalicylic acid in decreasing glycosuria and hyperglycemia in various animal preparations[3] at a dose level of about 500 mg/kg (*s.c.*) and in man[4] at about 5 g/day, is well established.

1) Part II: H. Asakawa, M. Matano and Y. Kawamatsu, *Chem. Pharm. Bull.* (Tokyo), **27**, 287 (1979).
2) Location: *Jusohonmachi, Yodogawa-ku, Osaka 532, Japan.*
3) *a*) V. Fang, W.O. Foye, S.M. Robinson and H.J. Jenkins, *J. Pharm. Sci.*, **57**, 2111 (1968); *b*) M.J.H. Smith, *Biochem. J.*, **52**, 649 (1952); *c*) G.C. Feeney, P. Carlo and P.K. Smith, *J. Pharmacol. Exp. Ther.*, **114**, 299 (1952); *d*) M.J.H. Smith, *Brit. J. Pharmacol.*, **10**, 110 (1955); *e*) M.J.H. Smith and B.W. Meade, *Biochem. J.*, **51**, 18 (1952).
4) *a*) D.J. Ingle, *Proc. Soc. Exp. Biol. Med.*, **75**, 673 (1950); *b*) J. Reid and A.I. Macdougall, *Brit. Med. J.*, ii, 1071 (1957); *c*) J. Reid, *Brit. Med. J.*, i, 897 (1959); *d*) A. Hecht and M.G. Goldner, *Metabolism*, **8**, 418 (1959); *e*) S.G. Gilgore, *Diabetes*, **9**, 392 (1960).

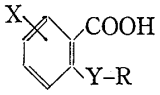


I

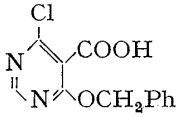
Recently, 1-(2-carboxyphenyl)pyrrole derivatives (I) were found to have hypoglycemic activity, and those having a chlorine atom *ortho* to the carboxy group (I, X=Cl) were generally more potent than their non-substituted counterparts (I, X=H).[5] The present paper describes the hypoglycemic activity of salicylic and anthranilic acid derivatives having a chlorine atom *ortho* to the carboxy group.

Table I summarizes the hypoglycemic activities of salicylic acid derivatives and related compounds. 6-Chlorosalicylic acid (**1**) and O-benzylsalicylic acid (**2**) had no hypoglycemic activity at a dose level of 100 mg/kg in eviscerated rats. However, comparison of the hypoglycemic activities of the O-benzyl-salicylic acids, **2**, **3**, and **4**, clearly shows the effect of the *ortho* chlorine atom. A chlorine atom at the *meta* position had no effect on the activity. The effects of positional difference are also seen in the O-allyl derivatives **9** and **10**. Other substituents at the *ortho* position increased the activity more or less, *e.g.* compounds **5**,**6** and **7**. However, in these cases activity was observed only in eviscerated rats, whereas the *ortho* chloro derivative (**3**) was active in both eviscerated and normal rats.

Table I. Hypoglycemic Activities of Salicylic Acid Derivatives and Related Compounds



1—16

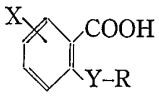
Compd. No.	R	Y	X	mp (°C)[a]	Hypoglycemic activity[b]	
					Eviscerated	Normal rats
1	H	O	6-Cl	170—171	0	
2	$PhCH_2$	O	H	73— 75	0	
3	$PhCH_2$	O	6-Cl	143—145	2	2
4	$PhCH_2$	O	5-Cl	114—116	0	
5	$PhCH_2$	O	6-OH	120	1	0
6	$PhCH_2$	O	6-MeO	104—106	2	0
7	$PhCH_2$	O	6-AcO	Oil	2	0
8	$PhCH(CH_3)$	O	6-Cl	Oil	2	
9	$CH_2=CH-CH_2$	O	5-Cl	80	0	
10	$CH_2=CH-CH_2$	O	6-Cl	Oil	2	
11		O	6-Cl	100	0	
12	$PhCH_2$	S	6-Cl	120—122	2	2
13	$PhCH_2$	SS	H	150—152	2	0
14		SS	H	105—106	2	0
15	*p*-Cl-$PhCH_2$	SS	H	182—183	0	
16	*p*-iso-Pr-$PhCH_2$	SS	H	140—147	0	
17				132—133	2	0

a) All melting points are uncorrected.
b) Reduction in blood glucose levels, calculated as a percentage decrease from the value of vehicle-treated control rats.
2: more than 40%, 1: 20—40%, 0: less than 20%.

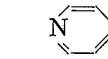
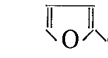
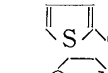
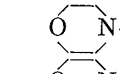
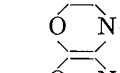
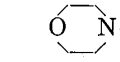
5) H. Sugihara, N. Matsumoto, Y. Hamuro and Y. Kawamatsu, *Arzneim.-Forsch.*, **24**, 1560 (1974).

As regards structure modification of the benzyloxy moiety, branching of the O-benzyl group (**8**) had no significant effect, at least when eviscerated rats were used. The activity disappeared in the compound having a tetrahydrofurfuryl group (**11**) in place of the benzyl group of **3**, but replacement of the ether oxygen with a sulfur atom (**12**) caused no change in the activity. In connection with this, it is interesting that the compound having a disulfide bond at this position (**13**) showed fairly strong hypoglycemic action even though the *ortho* chlorine atom was absent. The disulfide bond also activated the tetrahydrofurfuryl derivative (**14**); the *ortho* chlorine atom in its oxygen analog (**11**) did not exert any marked effect on the activity, as described above. Substitution at the *para* position of the benzyl group in **13** led to complete loss of activity, as shown by compounds **15** and **16**. A pyrimidine analog of **3**, compound **17**, showed some activity but it was weaker than that of the original compound.

Table II. Hypoglycemic Activities of Anthranilic Acid Derivatives and Related Compounds



18—30

Compd. No.	R	Y	X	mp (°C)[a]	Hypoglycemic activity[b]	
					Eviscerated	Normal rats
18	$PhCH_2$	NH	H	173—174	0	
19	$PhCH_2$	NH	6-Cl	159—160	2	1
20	*p*-MeO-$PhCH_2$	NH	6-Cl	122—122.5	0	
21	$PhCH_2CH_2$	NH	6-Cl	133—134	2	0
22	PhCO	NH	6-Cl	193—194	2	0
23	$PhCH_2CO$	NH	6-Cl	163—165	0	
24		NH	6-Cl	190—191	0	
25	PhCH=CH-CH_2	NH	6-Cl	115—116	0	
26		NH	6-Cl	192—193	0	
27		NH	6-Cl	185—186	0	
28		-N=	H	142—144	1	0
29		-N=	6-Cl	Oil	2	0
30		-N=	4-Cl	165—166	1	0

a) All melting points are uncorrected.
b) Reduction in blood glxcose levels, calculated as a percentage decrease from the value of vehicle-treated control rats.
2: more than 40%, 1: 20—40%, 0: less than 20%.

Table II lists the hypoglycemic activities of anthranilic acid derivatives and related compounds. Some similarities exist in the structure–activity relationships of anthranilic and salicylic acid derivatives. Comparison of the activities of **18** and **19** clearly shows the *ortho* chlorine effect. The *p*-methoxybenzyl derivative (**20**) had lost the activity. However, some structural modification of the benzyl group is permissible: Activity was observed for the compound having a phenethyl group (**21**) or benzoyl group (**22**) in place of the benzyl group in **19**, but was lost in the compound with a phenylacetyl group. Replacement of the benzene ring with pyridine (**24**) or of methylene with propenylene (**25**) in the benzyl moiety of **19**, also

had an undesirable effect on the activity. Similarly, replacements of the benzene ring in the benzoyl moiety of **22** with other heteroaromatic rings (**26** and **27**) result in loss of activity.

Another example of the effect of a chlorine atom *ortho* to the carboxy group is shown by the triazene derivatives (**28**—**30**). Here, the importance of the position of the chlorine atom is again clear.

These results and comparison of the data in Tables I and II led to the following conclusions:

1) The presence of a chlorine atom *ortho* to the carboxy group in salicylic and anthranilic acid derivatives may enhance the hypoglycemic activity.

2) O-Benzyl-6-chlorosalicylic acid (**3**) is more potent than N-benzyl-6-chloroanthranilic acid (**19**).

3) Modification of the benzyl group does not seem promising for improvement of the activity, but the hetero atom between the benzoic acid and benzyl moieties may be oxygen, sulfur or nitrogen.

Experimental

Hypoglycemic Activity——Male Sprague–Dawley rats aged 5 weeks were eviscerated as described by Russell.[6] These rats received 400 mg/kg of glucose subcutaneously at 1 hr intervals. The test compound was administered subcutaneously immediately after the operation and blood samples were taken from the tail vein 3 hr later. Blood glucose was determined by the method of Hoffman as adapted for the Technicon Autoanalyzer. In another test, performed as a second screening, normal fasted rats which had received glucose (1 g/kg *s.c.*) were employed. Test compounds were administered orally and blood samples were taken 2 and 4 hr later.

Preparation of Salicylic Acid Derivatives——2-Benzyloxybenzoic acid (**2**),[7] 2-benzyloxy-6-methoxybenzoic acid (**6**),[8] 2-benzylthio-6-chlorobenzoic acid and 2-substituted-dithiobenzoic acids (**12**—**16**)[9] were prepared according to the literature. 6-Chlorosalicyic acild (**1**) was obtained by hydrolysis of the 2-carboxy-3-chlorobenzenediazonium salt, which was itself derived from 6-chloroanthranilic acid by treatment with $NaNO_2$ and HCl. O-Substituted 6- or 5-chlorosalicylic acid derivatives (**3**, **4** and **8**—**11**) were prepared by alkylation of **1** or 5-chlorosalicylic acid with the appropriate halide in an alkaline medium. 2-Benzyloxy-6-hydroxybenzoic acid (**5**) was prepared by the partial benzylation of 2,6-dihydroxybenzoic acid with benzyl chloride, and its acetate (**7**) was obtained by the acetylation of **5** with acetic anhydride and pyridine. 4-Benzyloxy-5-carboxy-6-chloropyrimidine (**17**) was prepared from 5-bromo-4,6-dichloropyrimidine. A solution of Na (0.46 g) in benzyl alcohol (25 ml) was added portionwise to a solution of 5-bromo-4,6-dichloropyrimidine (4.56 g)[10] in tetrahydrofuran (THF) (100 ml) with stirring at room temperature. Next, the mixture was stirred for 1 hr and evaporated down under reduced pressure to remove the solvent. The residue was dissolved in water and the solution was extracted with ether, then the ether layer was dried over $MgSO_4$ and the ether was evaporated off. This residue was crystallized from EtOH, affording 4-benzyloxy-5-bromo-6-chloropyrimidine in 68% yield. A solution of the 4-benzyloxy compound (3.0 g) mentioned above in ether (50 ml) was added dropwise to a mixture of 10% *n*-BuLi in *n*-hexane (7.2 g) and ether (100 ml) with stirring at −40——35°. Dry ice (5 g) was added and the temperature was gradually raised to 0°. Next, water and 10% $NaHCO_3$ were added successively to the mixture, with stirring. The water layer was separated, acidified with conc. HCl and extracted with $CHCl_3$. The $CHCl_3$ layer was dried over $MgSO_4$ and evaporated down to remove the solvent. The residue was crystallized from ether–petroleum ether affording **17** (0.6 g, 23%). The structures of the prepared compounds were confirmed by elemental analyses (C, H or C, H, N).

Preparation of Anthranilic Acid Derivatives——N-Benzylanthranilic acid (**18**) was prepared according to the literature.[11] N-Substituted 6-chloroanthranilic acid derivatives (**20**—**25**) were prepared by the method described previously.[12] N-Benzyl-6-chloroanthranilic acid (**19**) was prepared by benzylation of 6-chloroanthranilic acid in an alkaline medium. N-Furoyl-6-chloroanthranilic acid (**26**) was obtained by treatment

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9) O. Aki, M. Numata and K. Shirakawa, *Takeda Kenkyusho Nempo*, **32**, 1 (1973).
10) J. Chesterfield, J.F.W. McOmie and E.R. Sayer, *J. Chem. Soc.*, **1955**, 3478.
11) J. Houben and W. Brassert, *Chem. Ber.*, **39**, 3233 (1960).
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of methyl 6-chloroanthranilate with furoyl chloride in the presence of pyridine, followed by hydrolysis of the ester. N-Thenoyl-6-chloroanthranilic acid (**27**) was prepared by a similar method using thionyl chloride.

Triazene derivatives (**28**—**30**) were prepared from the corresponding anthranilic acids. To a solution of anthranilic acid or chloroanthranilic acid (10 mmol) in 1 N HCl (40 mmol), a solution of $NaNO_2$ (20 mmol) in water (8 ml) was added with stirring at −5—0°. The resulting mixture was added portion wise to a solution of morpholine (20 mmol) and $NaCO_3$ (20 mmol) in water (50 ml) and this was stirred for 20 min. Next, 1 N HCl was added to the mixture and the precipitated solid was collected and crystallized from a suitable solvent, affording the triazene compounds (**28**—**30**).

The structures of these compounds were confirmed by elemental analyses (C, H, N).

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A Deblocking Method using Thioether–Sulfonic Acid Systems. Application to the Synthesis of Met-Enkephalin[1]

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Thioanisole–trifluoromethanesulfonic acid and thioanisole–methanesulfonic acid systems were found to be useful as deblocking methods in the synthesis of a methionine-containing peptide, Met-enkephalin, without any side reaction.

Keywords——peptide synthesis; thioanisole-trifluoromethanesulfonic acid; thioanisole-methanesulfonic acid; Met-enkephalin; deblocking method

Deblocking methods using trifluoromethanesulfonic acid (TFMSA)–trifluoroacetic acid (TFA)–anisole[3] and methanesulfonic acid (MSA)–anisole[4] were first described by Yajima and associates. Such procedures are complicated by the acidolytic cleavage of anisole, which, in the presence of methionine can result in the transfer of a methyl group from anisole to the sulfur atom of methionine.[5] This side reaction is usually prevented by conversion of methionine to the corresponding sulfoxide.[5,6]

We reasoned that replacement of anisole with a scavenger that was more acid-stable and a better cation acceptor might prevent the transmethylation reaction and therefore eliminate

1) Amino acids, peptides and their derivatives were all of L-configuration. The following abbreviations are used: Z=benzyloxycarbonyl, Bzl=benzyl, Cl_2Bzl=2,6-dichlorobenzyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, DCC=dicyclohexylcarbodiimide, ONP=*p*-nitrophenyl ester.

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4) H. Yajima, Y. Kiso, H. Ogawa, N. Fujii, and H. Irie, *Chem. Pharm. Bull.* (Tokyo), **23**, 1164 (1975).

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