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SYNTHESIS AND HYPOGLYCEMIC EFFECT OF CHRYSIN DERIVATIVES

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Abstracts A series of 18 chrysin derivatives, prepared by alkylation and condensation, were fully characterized by NMR and other techniques and tested *in vivo* against the diabetes mellitus. Several modified compounds especially those with propyl, butyl, octyl and tolyl groups were found to have hypoglycemic effect on diabetec mice in spite of the fact that chrysin itself had inhibited insulin release by 40-60%. None of the test animals died at the maximum dose 500mg/kg and did not cause any significant change in general feature, water and food consumption, body weight and organ weight when we examined the acute oral toxicity of those compounds having significant hypoglycemic effect
Keywords : chrysin, hypoglycemic effect, diabetes, streptozotocin, acute oral toxicity

Introduction

Chrysin is a flavone widely distributed in plants which was reported to have many different biological activities including anti-oxidant[1], anti-allergic[2], anti-inflammatory[3], anti-cancer[4-5], antiestrogenic[6] and anxiolytic[7] activities. However, chrysin or its derivatives have not been reported to have hypoglycemic effect. Furthermore, chrysin(0.08mmol/l) was found to inhibit insulin release by approximately 40-60%.[8] In this study, we synthesized chrysin derivatives and examined their effects on the blood glucose level in order to evaluate their anti-diabetogenic activity using streptozotocin(STZ) as a diabetogenic agent.[9-10] And also we examined the acute oral toxicity of 4 compounds.

Chemistry

Chrysin alkyl derivatives were prepared by alkylation using alkyl bromide or alkyl chloride. As a result, 5,7-dialkoxychrysin and 5-hydroxy-7-alkoxychrysin were synthesized(scheme 1). In case of monoalkyl derivatives, various analytical data, especially NOESY and COLOC spectra showed that substitutions occurred mostly at the 7-position of chrysin but very little at its 5-position.[11] The reason why the substitution occurred mostly at the 7 position may be due to the hydrogen bonding of 5-OH group with carbonyl group. When acetic anhydride was reacted with chrysin, only a diacetyl derivative was produced(scheme 2). Only 5-acetoxy-7-hydroxychrysin, but not 7-acetoxy-5-hydroxychrysin was obtained by partial hydrolysis of the diacetyl derivative

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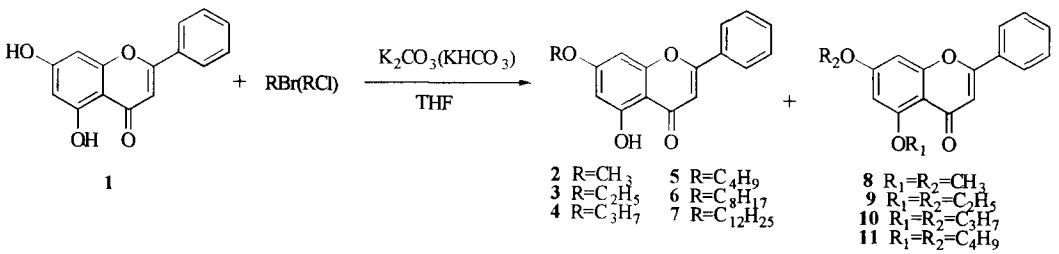
in MeOH or EtOH. It is supposed that 7-position of 5,7-diacetoxychrysin was easily attacked during the hydrolysis because it was open. Chrysin acyl derivatives were synthesized by condensation using dicyclohexylcarbodiimide(DCC) and 4-dimethylaminopyridine(DMAP) in THF solvent or condensation using diethylphosphoryl cyanide (DEPC) and triethylamine(TEA) in DMF solvent(scheme 3). In this case, chrysin derivatives substituted only at the 7-position were formed, but not 5-position.[12]

Scheme 1

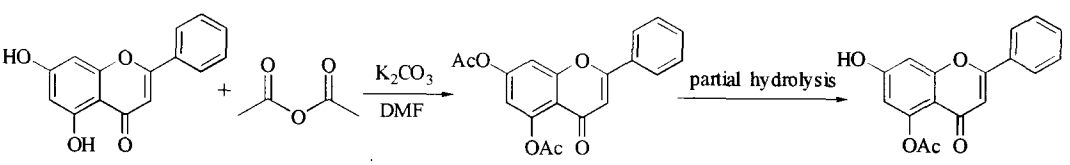


1

2 R=CH_3 5 R=C_4H_9
3 R=C_2H_5 6 R=C_8H_{17}
4 R=C_3H_7 7 R=$C_{12}H_{25}$

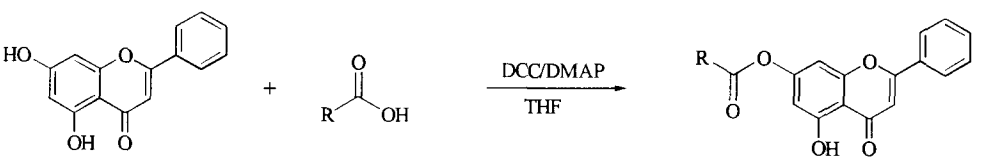
8 R_1=R_2=CH_3
9 R_1=R_2=C_2H_5
10 R_1=R_2=C_3H_7
11 R_1=R_2=C_4H_9

Scheme 2



12 13

Scheme 3



14 R=Acetylsalicylyl
15 R=Benzyl
16 R=Cinnamyl
17 R=4-Methoxycinnamyl
18 R=*p*-Tolyl
19 R=*p*-Fluorophenyl

Biological Activity

Streptozotocin(45 mg/kg bodyweight, 0.01M citrate buffer)[13-14] was injected i.v. into the tail of the male SD rats. After 24 hours, blood was collected from the eye-ball veniplex of the rats and the serum was obtained by centrifugation of the blood. When glucose concentration was more than 300 mg/dl in the serum, the rat was selected as a diabetec. The compounds were suspended in 3% Tween 80(40-50mg/kg bodyweight). A dose of 1mg/100g bodyweight was orally administered to the rats once daily for seven days. We started administration of

samples immediately after we confirmed the rat as the diabetec. On 24 hours after the last administration of the dose, the rats were anesthetized and the blood was attained from the heart. After the blood was collected from the heart in heparinized tubes, the serum was separated by centrifugation. Glucose concentration in the sera was measured by using a glucose kit based on glucose oxidase method.[15] After mean values and standard deviations were calculated, the values of the control and treated groups were subjected to F-test. The significance of the differences between those groups was evaluated according to LSD test.

The acute oral toxicity of compounds 5, 6, 10 and 18 in rats and mice

The acute oral toxicity of 4 substances, **5, 6, 10** and **18** were examined in male SD rats and male ICR mice. The test substances were suspended in 1% CMC. A single dose of 500mg/kg was orally administered into the rats and doses of 5mg/kg, 20mg/kg, 100mg/kg, 500mg/kg into the mice. For the control group only 1% CMC was administered. The suspensions of the test substances were administered at a volume of 20ml/kg

Table 1. Properties and *in vivo* activities of chrysin derivatives.

Group	m.w.	Yield (%)	m.p. (℃)	UV (λ_{max}^{MeOH})	Dose (mg/kg)	No. of animal	Blood glucose level(mg/dl)		7day/0day (%)
							0 day	7 day	
control	—	—	—	—	—	5	461.2±122.8	400.0±78.8	86.7
1	254	—	285-286	269, 312	45	7	482.4±46.4	503.5±62.5	104.4
2	268	40	163-166	268, 307	50	5	455.0±33.6	399.6±85.9	87.8
3	282	30	143-149	268, 310	50	5	400.4±55.0	405.4±34.7	101.2
4	296	15	122-125	269, 301	50	5	400.0±19.4	237.8±115.2*	59.5
5	310	41	137-141	268, 311	50	5	545.6±79.1	252.4±124.7*	46.3
6	366	95	80-86	269, 311	50	5	411.2±41.9	211.4±64.2*	51.4
7	422	95	89-92	257, 308	50	5	465.6±45.3	381.3±35.5*	81.9
control	—	—	—	—	—	6	449.2±61.2	486.3±100.0	108.3
8	282	42	143-145	264, 306	40	8	444.4±49.8	399.5±83.0	89.9
9	310	52	145-148	264, 307	45	8	436.6±62.5	400.5±89.7	91.7
10	338	40	127-129	264, 306	42	7	448.3±69.9	287.0±141.1*	64.0
11	366	20	108-110	264, 307	43	8	454.4±82.8	405.7±146.8	89.3
12	338	95	198-201	254, 294	43	7	472.0±60.5	341.5±124.8*	72.4
13	296	95	162-164	268, 311	—	—	—	—	—
control	—	—	—	—	—	9	418.4±37.3	413.6±43.1	98.9
14	416	14	193-198	271, 294, 329	40	6	489.3±98.2	500.9±124	102.4
15	358	97	185-191	226, 269, 326	50	5	496.2±37.4	515.6±44.3	103.9
16	384	73	180-182	278, 362	50	5	440.6±60.3	407.9±38.0*	92.6
control	—	—	—	—	—	4	447.7±120.5	356.2±147.7	79.6
17	414	92	218-221	273, 318	40	6	475.9±69.8	333.3±177.3	70.0
18	376	97	192-196	229, 268, 327	40	5	464.3±89.2	261.8±157.5*	56.4
19	372	96	190-192	268,328	40	5	458.2±40.6	363.5±182.5	79.3

(Values are mean ± S.D., significantly different from the control * $p < 0.05$)

bodyweight. The rats and mice were housed conventionally under barrier conditions for 1 week in polycarbonate cages. The room temperature was maintained at 23 ± 2 °C and the relative humidity at 55 ± 10%. Artificial light was provided continuously for 12 hr/day. Food and water were freely provided. However, prior to dosing (since 15 hours before dosing), the rats and mice were fasted. All experiments were carried out on 10 a.m. The test animals were observed every hour for 6 hours soon after dosing. On subsequent days the animals were observed for 1 hour once in a day for 12 days. Changes of general feature, special symptoms, mortalities, food intake and water intake were observed. Individual bodyweights of all the animals were recorded on 3rd, 6th, 9th and 12th day prior to and after dosing. On 13th day after dosing, the rats were killed by exsanguination from the abdominal aorta while they were under light ether anesthesia. The morphology of the external and internal organs were carefully examined. And the weights of liver, spleen, kidney and lung were measured. The data were evaluated by ANOVA test and Neuman-Keuls test.

Table 2. Acute oral toxicity data of chrysin 4 derivatives. (compound **5, 6, 10** and **18**)

Animal	Treatment (mg/kg)		Morta-lity[a]	Signs[b]	Water con -sumption[c]	Food con -sumption[d]	Body weight[e]		Organ weight[f] (Rat)	
							0 day	12 day	Liver	
Mouse	Control		0/10	-	82.92	52.88	26.2 ± 1.69	33.5 ± 1.84	Con.	10.66 ± 0.32
	5	5	0/10	-	82.50	51.91	24.9 ± 1.60	32.7 ± 2.54	**5**	10.74 ± 1.33
		20	0/10	-	79.33	52.81	23.8 ± 1.23	30.6 ± 3.09	**6**	9.96 ± 1.28
		100	0/10	-	80.92	50.53	23.5 ± 1.84	31.0 ± 3.17	**10**	9.64 ± 0.70
		500	0/10	-	82.83	50.40	24.3 ± 2.31	31.3 ± 2.15	**18**	9.60 ± 0.90
	6	5	0/10	-	79.83	51.28	23.8 ± 2.62	30.8 ± 2.70	Spleen	
		20	0/10	-	81.67	52.57	25.5 ± 2.46	32.1 ± 2.76	Con.	0.63 ± 0.07
		100	0/10	-	82.83	51.41	24.8 ± 1.93	31.6 ± 2.92	**5**	0.71 ± 0.07
		500	0/10	-	80.75	50.60	24.0 ± 2.54	30.1 ± 2.61	**6**	0.69 ± 0.09
	10	5	0/10	-	80.00	48.09	22.3 ± 1.89	28.2 ± 1.65	**10**	0.61 ± 0.06
		20	0/10	-	80.92	53.20	25.3 ± 3.16	31.9 ± 3.51	**18**	0.66 ± 0.07
		100	0/10	-	81.75	49.77	25.2 ± 2.44	32.1 ± 2.45	Kidney	
		500	0/10	-	81.75	49.87	22.7 ± 2.54	29.5 ± 1.58	Con.	0.93 ± 0.04
	18	5	0/10	-	80.42	49.35	25.1 ± 1.97	32.2 ± 1.92	**5**	1.04 ± 0.07
		20	0/10	-	80.75	50.60	24.7 ± 2.50	31.4 ± 2.29	**6**	1.01 ± 0.09
		100	0/10	-	83.75	50.78	25.2 ± 2.70	31.6 ± 2.72	**10**	0.97 ± 0.06
		500	0/10	-	82.08	49.86	24.8 ± 2.44	31.2 ± 3.55	**18**	0.98 ± 0.07
Rat	Control		0/6	-	218.16	116.50	236.7 ± 9.3	301.7 ± 14.7	Lung	
									Con.	1.22 ± 0.06
	5	500	0/6	-	197.16	113.58	235.0 ± 8.4	293.3 ± 7.5	**5**	1.19 ± 0.07
	6	500	0/6	-	205.91	113.08	230.8 ± 8.0	290.8 ± 18.8	**6**	1.26 ± 0.09
	10	500	0/6	-	218.50	115.41	235.8 ± 4.9	300.8 ± 8.6	**10**	1.23 ± 0.04
	18	500	0/6	-	216.83	112.83	237.5 ± 8.2	302.5 ± 5.2	**18**	1.26 ± 0.20

a : Mortality of experimental animals orally treated with compound **5,6,10** and **18**. Each value represents No. of dead animals per No. of treated animals until 12th day. **b** : Clinical signs in experimental animals orally treated with compound **5,6,10** and **18**. (-) represents negative sign of abnormal symptoms in treated animals. **c** : Daily water consumption in mice and rats orally treated with compound **5,6,10** and **18**. Each value represents an average (ml) of daily water consumption of 10 mice and 6 rats for 12 days. **d** : Daily food consumption in mice and rats orally treated with compound **5,6,10** and **18**. Each value represents an average (g) of daily food consumption of 10 mice and 6 rats for 12 days. **e** : Bodyweights in mice and rats orally treated with compound **5,6,10** and **18**. Each value represents mean ± S.D. of 10 mice and 6 rats in each cage(this table have only values of 0 and 12th day). **f** : Organ weights of SD-rats(treatment: 500mg/kg) orally treated with compound **5,6,10** and **18**. Each value represents mean ± S.D. of 6 rats in a cage.

Results and discussion

Properties and hypoglycemic activities of chrysin derivatives were shown in **Table 1**. When injected into the mice treated with streptozotocin, several compounds elicited hypoglycemic effect. Among them, **4**, **5**, **6**, **7**, **10**, **12**, **16** and **18** decreased the glucose level in the blood as compared with that of the control. Particularly compound **5** showed more than 50% decrease in the blood glucose level at day-7 in comparison with that of day-0. This fact is remarkable when it was compared with the result of the effect of the parent compound chrysin on diabetes. And for the first time we revealed that chrysin derivatives have a hypoglycemic effect. Although general structure-activity relationship of the chrysin derivatives to hypoglycemic effect was not elucidated from these data, the alkyl derivatives mostly exhibited stronger activity than the acyl derivatives and among the alkyl derivatives, the compounds with 3, 4 and 8 carbons showed significant activity. And it was obvious that hydroxyl group of the 5-position was important for activity when compound **4** and **5** were compared with the results of the effect of the compound **10** and **11**. Although most of the acyl derivatives did not show significant activity as compared with that of the control group, compound **18** with the methyl group at the *para* position of the benzene ring exhibited significant activity.

The acute oral toxicity of the test substances was shown in **Table 2**. None of the test animals died at the maximum dose 500mg/kg during the experiment. When the animals were observed, no change in the organs was detected in all the animals. The administrations of the samples did not cause any change in the uptake amount water and feeds as compared with that of the normal control group. The increase of bodyweight after administration was not significant when the weights of the normal control group were compared. When the morphology of the external and internal organs of the scarified rats were examined, no abnormal change was detected. When the weights of liver, spleen, kidney and lung were measured, the test substances did not cause any significant change in them. A more detailed analysis of SAR and further work for the toxicity and biological activities will be described in future publications.

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11. For example, NOESY spectra of compound **3** showed that CH_2 signal at δ 4.12 resulted in a positive NOE signal corresponding to H-6 at δ 6.36 and H-8 at δ 6.49. And also, COLOC spectra showed the long-range coupling of the 5-OH signal (at δ 12.70) with C-5 (at δ 162.57)and C-10(at δ 106.02) positions.

12. All the new compounds were well characterized by IR, UV, Mass, ^{1}H-NMR, ^{13}C-NMR, ^{1}H-^{1}H COSY, ^{13}C-^{1}H COSY, DEPT, NOESY and COLOC analysis. **3**: Mass(EI,70eV) m/e 282. ^{1}H-NMR(300MHz,$CDCl_3$): δ 1.374-1.391(t,J=2.55,3H), 4.022- 4.082(q,2H), 6.296-6.302(d,J=1.8Hz,1H), 6.422-6.428(d,J=1.8Hz,1H), 6.599(s,1H), 7.438-7.472(m,3H), 7.808-7.831(m,2H), 12.64(s,1H). ^{13}C-NMR(300MHz,$CDCl_3$): δ 14.98(CH_3), 64.63(CH_2), 93.50(CH), 98.98(CH), 106.02 (C), 106.28(CH), 126.69(2CH), 129.48(2CH), 131.80(C), 132.19(CH), 158.21(C), 162.57(C), 164.34(C), 165.43(C), 182.88(C). IR V_{max} (cm^{-1}, KBr) : 3405(OH), 1661(C=O) **9**: Mass(EI,70eV) m/e 310. ^{1}H-NMR (300MHz,$CDCl_3$): δ 1.434-1.480(t,J=6.9,3H), 1.514-1.560(t,J=6.9,3H), 4.074-4.173(m, 4H), 6.343-6.351(d,J=2.1Hz,) 1H), 6.518-6.525(d,J=2.1Hz,1H), 6.889(s,1H), 7.466-7.502(m,3H), 7.837-7.870(m,2H). ^{13}C- NMR(300MHz,$CDCl_3$): δ 14.91(CH_3), 15.00(CH_3), 64.51(CH_2), 65.41(CH_2), 93.60(CH), 97.85(CH), 109.49(CH), 109.83(C), 126.35(2CH), 129.31(2CH), 131.49(CH), 132.09(C), 160.31(C), 160.64(C), 160.90(C), 163.73(C), 177.99(C). IR V_{max} (cm^{-1}, KBr) : 1647(C=O) **12**: Mass(EI,70eV) m/e 338. ^{1}H-NMR(300MHz,$CDCl_3$): δ 2.291(s,3H), 2.382(s,3H), 6.599(s,1H), 6.780 6.786(d,J=2.4Hz,1H), 7.294-7.300(d,J=2.4Hz, 1H), 7.450-7.486(m,3H), 7.784-7.805(m,2H). ^{13}C- NMR(300MHz,$CDCl_3$): δ 21.51(CH_3), 21.60(CH_3), 109.00(C), 109.47(CH), 114.07(CH), 115.37(CH), 126.62(2CH), 129.50(2CH), 131.46(C), 132.20(CH), 150.62(C), 154.34(C), 158.10(C), 162.98(C), 168.43(C), 169.85(C), 176.83(C). IR V_{max} (cm^{-1}, KBr) :1770 (C=O), 1646(C=O) **13**: Mass(EI,70eV) m/e 296. ^{1}H- NMR(300MHz,$CDCl_3$): δ 2.272(s,3H), 6.468-6.475(d,J=2.1Hz, 1H), 6.695(s,1H), 6.826-6.833(d, J=2.1Hz,1H), 7.547-7.588(m,3H), 7.995-8.069(m,2H). ^{13}C-NMR(300MHz,$CDCl_3$): δ 21.86(CH_3), 101.76(CH), 108.25(2CH), 110.16(C), 126.97(2CH), 129.95(2CH), 131.87(C), 132.38(CH), 150.94(C), 159.34(2C), 161.50(C), 169.72(C), 176.03(C). IR V_{max} (cm^{-1}, KBr) : 3402(OH), 1652(C=O) **16**: Mass(EI,70eV) m/e 384. ^{1}H-NMR(300MHz,$CDCl_3$): δ 6.615-6.654(d,J=15.9Hz,1H), 6.670-6.675(d,J=2.0Hz,1H), 6.750(s,1H), 6.965-6.970(d, J=2.0Hz,1H), 7.441-7.458(m,3H), 7.538-7.562(m,3H), 7.603-7.627(m,2H), 7.895-7.936(m,3H), 12.75(s,1H). ^{13}C-NMR (300MHz,$CDCl_3$): 101.49(CH), 105.93(CH), 106.57(CH), 109.32(C), 116.90(CH), 126.84(2CH), 128.86(2CH), 129.49 (2CH), 129.59(2CH), 131.43(C), 131.47(CH), 132.56(CH), 134.31(C), 148.13(CH), 156.60(C), 157.21(C), 162.34(C), 164.76(C), 165.12(C), 183.31(C). IR V_{max} (cm^{-1},KBr) :1734(C=O)

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