The Hypoglycemic Effect of (*7R*,9aS**)-7-Phenyl-octahydroquinolizin-2-one in Mice

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(−)-Multiflorine (1), which was isolated from leguminous plants, produced a hypoglycemic effect when administered to mice with streptozotocin-induced diabetes. (−)-Multiflorine has an enaminone type conjugation on the A-ring, which is unusual in lupine alkaloids. Proceeding on the assumption that the A–B ring is responsible for the activity, several compounds bearing quinolizidin-2-one were synthesized and their hypoglycemic effects were examined. The hypoglycemic effect of (*7R*,9aS)-7-phenyl-octahydroquinolizin-2-one was approximately 4 times stronger than that of (−)-multiflorine measured by oral glucose tolerance test in normal mice. This result indicates that compounds possessing the quinolizidin-2-one ring system as the basic structure may be possible lead compounds for a new type of diabetes drug.**

Key words quinolizin-2-one; multiflorine; hypoglycemic effect; lupine alkaloid

Of the hypoglycemic agents that have been used clinically, biguanides[1,2] and sulfonylureas[3,4] have pharmacological activity for non-insulin dependent diabetes mellitus (NIDDM), but they are ineffective for insulin dependent diabetes mellitus (IDDM). β-Glucosidase inhibitors are useful as adjuncts to dietary therapies. Although insulin is the most effective drug for both types of diabetes, oral administration is not recommended. Thus, efficient drugs for the treatment of both types of diabetes are desired.

(−)-Multiflorine (**1**)[5] is a lupine alkaloid that is known to have hypoglycemic activity when administerd to mice with streptozotocin-induced diabetes.[6] This indicated that multiflorine-like compounds are effective for IDDM. (−)-Multiflorine (**1**) has been isolated from *Lupinus hirsutus*[7] and *Lupinus termis* (Leguminosae). In Greece, *L. hirsutus* is called "agriolupino" and is used as a traditional drug for treatment of diabetes. The seeds of *L. termis* are a popular food in Egypt after the alkaloids have been removed by water-immersion for several days.

Our study focused on discovering the basic structure of non-insulin dependent hypoglycemic agents using (−)-multiflorine (**1**) as a lead compound. Because we expected that the hypoglycemic effect was caused by the A/B ring system of (−)-multiflorine, several compounds (**2**—**8**) bearing the quinolizin-2-one ring system were synthesized and their hypoglycemic effects were evaluated.

We first examined the hypoglycemic effect of the synthetic compounds in STZ-induced diabetic mice. Compounds that produced a hypoglycemic effect in diabetic mice were then evaluated by oral glucose tolerance tests in normal mice.

MATERIALS AND METHODS

Animals Male ICR mice (Tokyo Laboratory Animals Science, Tokyo, Japan), weighing about 30 g (6 weeks old) were used. The mice had free access to food and water in an animal room that was maintained at 24±1 °C with a 12 h light–dark cycle. The mice were fasted for 12 h before the oral glucose tolerance test. Studies were carried out in accordance with the Declaration of Helsinki and/or with the Guide for the Care and Use of Laboratory Animals as adopted by the Committee on the Care and Use of Laboratory Animals of Hoshi University, which is accredited by the Ministry of Education, Science, Sports and Culture.

Induction of Diabetes by Streptozotocin Animals were rendered diabetic by injection of streptozotocin (STZ; 200 mg/kg, i.v.) prepared in 0.1 M citrate buffer at pH 4.5 under light ether anesthesia. The experiments were conducted 2 weeks after injection of STZ. Mice with serum glucose levels above 400 mg/dl were considered diabetic.

Plant Material and Isolation of Lupine Alkaloids The seeds of *Lupinus termis* L. (1.0 kg) were bought in Egypt in 1998. The dry *L. termis* seeds were extracted with 75% MeOH three times at room temperature. The aqueous concentrate was acidified with 10% HCl to pH 3 and the resulting precipitate was filtered out. The filtrate was extracted three times with CH_2Cl_2. The aqueous layer was made strongly alkaline with K_2CO_3 and extracted three times with CH_2Cl_2. The CH_2Cl_2 layers were combined, dried over Na_2SO_4, and concentrated *in vacuo* to give a crude base (18.2 g, yield 1.8%). The crude base was separated by chromato-

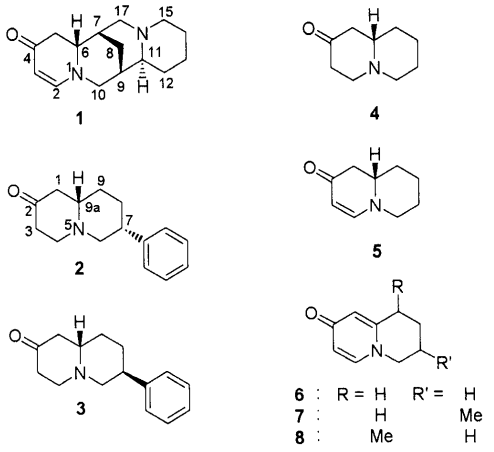

Fig. 1

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graphy on Si-gel with a solvent gradient of CH_2Cl_2–MeOH–25% NH_4OH. The eluted volume of each fraction was 100 ml.

Isolation of (−)-Multiflorine (1) The crude alkaloid mixture was separated by silica gel column chromatography and purified by preparative HPLC to give (−)-multiflorine. (−)-Multiflorine (**1**): colorless needles. mp 108 °C $[\alpha]_D$ −271.1° (c=0.381, MeOH). IR ν (cm^{-1}): 2940, 2850 (Bohlmann bands), 1630 (conjugated C=O), 1580 (conjugated –HC=CH–). EI-MS *m/z* (rel. %): 246 ([M^+], 78), 189 (11), 164 (10), 149 (30), 136 (33), 134 (100), 110 (30), 97 (38), 83 (41), 69 (66), 55 (48), 41(44). ^{13}C-NMR ($CDCl_3$) δ: 192.5 (s) 155.6 (d) 98.9 (d), 63.6 (d), 60.3 (d), 57.5 (t), 55.2 (t), 51.1(t), 39.3 (t), 34. 5(d), 31.5 (t), 31.1(d), 25,8 (t), 24.8 (t), 23.7 (t). ^{1}H-NMR ($CDCl_3$, 270 MHz) δ: 6.84 (1H, d, J=7.7 Hz), 4.96 (1H, d, J=7.7 Hz), 3.46 (1H, ddd , J=15.9, 5.2, 2.5 Hz), 3.19 (1H, dm, J=12.1 Hz), 3.14 (1H, dd, J=12.1, 3.0 Hz), 3.07 (1H, m), 2.92 (1H, dd, J=11.8, 8.8 Hz), 2.81 (1H, dt, J=12.1, 1.8 Hz) 2.68 (1H, t, J=16.2 Hz), 2.37 (1H, dd, J=11.8, 3.5 Hz), 2.3—2.2 (3H, m), 2.06 (1H, ddd, J=12.9, 3.6, 3.6 Hz), 1.86 (1H, dd, J=15.7, 1.9 Hz), 1.78 (1H, dm, J=12.2 Hz), 1.7—1.5 (4H, m) 1.4—1.2 (2H, m).

Synthesis of (*7R*,9aS)-7-Phenyl-octahydroquinolizin-2-one and (*7S*,9aS**)-7-Phenyl-octahydroquinolizin-2-one** Following the procedure outlined by King,[8] two compounds were synthesized.

(*7R*,9aS)-7-Phenyl-octahydroquinolizin-2-one (2)** Yield: 44.5% from starting material, phenylacetonitrile. Colorless crystals. ^{1}H-NMR ($CDCl_3$) δ: 7.55 (2H, d, J=7.3 Hz), 7.2—7.3 (3H, m), 3.23 (2H, m), 3.15 (1H, m), 2.67 (1H, m), 2.6—2.4 (4H, m), 2.3—2.2 (2H, m), 1.83 (2H, m), 1.53 (2H, m).

(*7S*,9aS)-7-Phenyl-octahydroquinolizin-2-one (3)** Yield: 18.5% from starting material, phenylacetonitrile. Pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 7.25 (5H, m), 3.15 (2H, m), 2.90 (2H, m), 2.73 (1H, m), 2.42 (3H, m), 2.26 (2H, m), 2.0—1.7 (2H, m), 1.65 (2H, m).

(±)-Octahydroquinolizin-2-one (4) To a solution of 4-methoxy-2-picoline in anhydrous THF was added 1.4 M *n*-BuLi at −40 °C. The resulting solution was stirred at −30 °C for 1 h. 1-Iodo-3-chloro-propane was added, and the solution was stirred for 8 h. Water was added, and the solution was extracted with CH_2Cl_2 three times. The organic layers were combined, dried with Na_2SO_4, and concentrated. Column chromatography on silica gel gave 4-methoxy-2-(4-chlorobutyl)-pyridine. This compound was dissolved in EtOH and refluxed for 10 h. EtOH was removed *in vacuo*, and the residue was washed with hexane to give a crude quaternary amine salt. The crude salt (360 mg, *ca.* 1.9 mmol) was dissolved in MeOH (12 ml), $NaBH_4$ (650 mg, 17.1 mmol) was added, and the solution was stirred 36 h at room temperature. Saturated K_2CO_3 (2.5 ml) was added and the solution extracted with CH_2Cl_2, dried over Na_2SO_4, and the solvent was removed *in vacuo* to give a crude enol ether. This enol ether was dissolved in 10% HCl and stirred for 8 h at room temperature. After hydrolysis, the solution was made alkaline with K_2CO_3 and extracted with CH_2Cl_2. The solvent was removed *in vacuo*; the residue was separated by chromatography on Si-gel to give (±)-octahydroquinolizin-2-one (4).[9] Yield 21.4% from 4-methoxy-2-picoline. Pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 3.2—2.9 (2H, m), 2.8—2.6 (1H, m), 2.4—2.2 (3H, m), 2.1—2.0 (2H, m), 1.8—1.6 (4H, m), 1.5—1.1 (3H, m).

(±)-1,6,7,8,9,9a-Hexahydroquinolizin-2-one (5) To a solution of 4-methoxypyridine (10 mmol) in fresh dry THF (40 ml), carbobenzoxy chloride (5.92 ml, 10 mmol) was added and stirred under nitrogen atmosphere at −60 °C for 1 h. Grignard reagent (4-chlorobutyl magnesium bromide, 10 mmol in THF) was added to the above THF solution and stirred. After 1 h, the solution was warmed to room temperature. Ice (20 g) and concentrated-HCl (2.5 ml) were added, and the solution was stirred for 8 h. The reaction mixture was extracted with CH_2Cl_2 and dried over Na_2SO_4. Solvent was removed *in vacuo* and the residue was purified by chromatography on Si-gel to give 2-(4-chlorobutyl)-4-oxo-3,4-dihydro-2*H*-pyridine-1-carboxylic acid benzyl ester (1.41 g, yield 44%).[10] This compound (440 mg, 1.36 mmol) was dissolved in EtOAc (20 ml) and hydrogenated with 10% Pd–C (50 mg) as a catalyst at atmospheric pressure for 8 h. The catalyst was removed by filtration, and solvent was removed *in vacuo* to give crude (±)-1,6,7,8,9,9a-hexahydroquinolizin-2-one. The crude compound was purified by Si-gel chromatography to give (±)-1,6,7,8,9,9a-hexahydroquinolizin-2-one (**5**, 170 mg, yield 66%).

Pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 6.87 (1H, d, J=7.3 Hz), 4.98 (1H, d, J=7.3 Hz), 3.41 (1H, m), 3.26 (1H, m), 2.99 (1H, ddd, J=12.2, 3.0, 3.0 Hz), 2.46 (1H, dd, J=16.4, 6.1 Hz), 2.36 (1H, dd, J=16.4, 12.8 Hz), 2.0—1.3 (6H, m).

Preparation of 6,7,8,9-Tetrahydroquinolizin-2-ones 1.4 M *n*-BuLi was added to a solution of 4-methoxy-2-picoline in anhydrous THF at −40 °C. The resulting solution was stirred at −30 °C for 1 h. 1-Iodo-3-chloro-propanes were added to the solution and stirred for 8 h. Water was added, and the solution was extracted with CH_2Cl_2 three times. The organic layers were combined, dried with Na_2SO_4, and concentrated. Column chromatography on silica gel gave 4-methoxy-2-(4-chlorobutyl)-pyridines. 4-Methoxy-2-(4-chlorobutyl)-pyridines were dissolved in EtOH and refluxed for 10 h. EtOH was removed *in vacuo* and the residue was washed with hexane to give a crude quaternary amine salt. The crude salts were refluxed in toluene for 10 h and toluene was removed. Quinolizin-2-ones were purified by column chromatography on Si-gel.

6,7,8,9-Tetrahydroquinolizin-2-one (6) Yield: 21.4% from 4-methoxy-2-picoline. Pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 7.24 (1H, d, J=7.9 Hz), 6.37 (1H, dd, J=7.9, 2.4 Hz), 6.27 (1H, d, J=2.4 Hz), 3.90 (2H, dd, J=6.1, 6.1 Hz), 2.79 (2H, dd, J=6.7, 6.7 Hz), 2.0—1.8 (4 H, m).

(±)-7-Methyl-6,7,8,9-tetrahydroquinolizin-2-one (7) Yield: 63.8% from 4-methoxy-2-picoline. Pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 7.24 (1H, d, J=7.4 Hz), 6.34 (1H, dd, J=7.4, 2.6 Hz), 6.27 (1H, d, J=2.6 Hz), 3.87 (1H, dd, J=12.5, 5.0 Hz), 3.46 (1H, dd, J=12.5, 10.2 Hz), 2.80 (2H, m), 2.1—1.9 (2H, m), 1.5—1.3 (1H, m), 1.10 (3H, d, J=6.6 Hz).

(±)-9-Methyl-6,7,8,9-tetrahydroquinolizin-2-one (8) A solution of compound **6** (100 mg, 0.67 mmol) in dry THF (5 ml) and HMPA (0.1 ml) was cooled to −50 °C and *n*-BuLi (0.5 ml, 0.8 mmol) was added to the solution and stirred for 1 h. MeI (113 mg, 0.8 mmol) was added to the solution and stirred for 3 h. The reaction was quenched with water and ex-

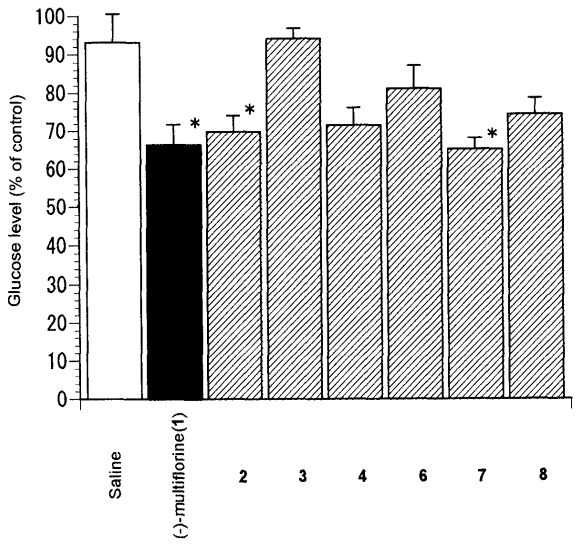

Fig. 2. Hypoglycemic Effect in STZ-Induced Diabetic Mice

$*p<0.05$ *vs.* vehicle (saline or 0.5% CMC) treated group.

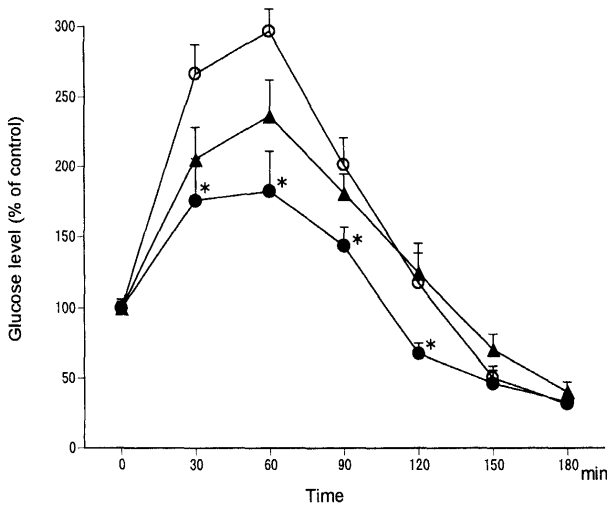

Fig. 3. Effect of (−)-Multiflorine (**1**) (●; 40 mg/kg, i.p., ▲; 20 mg/kg, i.p.) on the Blood Glucose Level of Normal Mice in the oral Glucose Tolerance Test

Each value represents the mean with S.E. ($n=10$). $*p<0.05$ *vs.* vehicle (saline; ○) treated group.

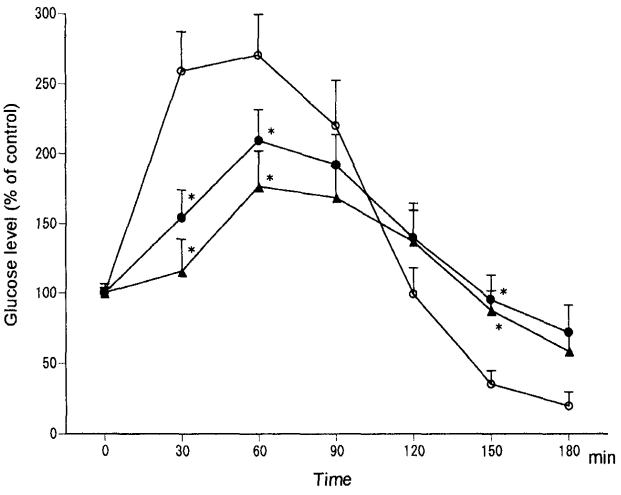

Fig. 4. Effect of Compound **2** (●; 3 mg/kg, *i.p.*, ▲; 10 mg/kg, i.p.) on the Blood Glucose Level of Normal Mice in the Oral Glucose Tolerance Test

Each value represents the mean with S.E. ($n=10$). $*p<0.05$ *vs.* vehicle (0.5% CMC; ○) treated group.

tracted with CH_2Cl_2 three times. The organic layers were combined, dried with Na_2SO_4, and concentrated. Column chromatography on silica gel gave (±)-9-methyl-6,7,8,9-tetrahydroquinolizin-2-one (**8**). Yield: 76.7%. Pale yellow crystals. mp 54 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.27 (1H, d, J=7.4 Hz), 6.37 (1H, d, J=2.6 Hz), 6.34 (1H, dd, J=7.4, 2.6 Hz), 3.9—3.8 (2H, m), 2.8 (1H, m), 2.0—1.5 (4H, m), 1.33 (3H, d, J=6.8 Hz).

Hypoglycemic Assay For screening of STZ-induced diabetic mice, each mouse was injected intraperitoneally with the test drugs (40 mg/kg). The blood was collected *via* caudalis venipuncture before the test and 30, 60, 90 and 120 min after the injection. The blood glucose level was measured by automatic blood glucose level measurement (Daikin). The reduction ratio of the blood glucose level was calculated for each animal using the formula: 100×(post-drug blood glucose level)/(pre-drug blood glucose level). Compounds that yielded values significantly different from those of vehicle (saline or CMC) after 60 min, were examined further for hypoglycemic effects in normal mice.

The hypoglycemic effect was evaluated by glucose tolerance test. Each mouse was injected *i.p.* with the test drugs, followed by *p.o.* with a glucose solution (3.0 g/kg). Blood was collected *via* caudalis venipuncture before the test and 30, 60, 90, 120, 150, and 180 min after the injection. The increment ratio of the blood glucose level was calculated for each animal using the formula: 100×(post-drug blood glucose level−pre-drug blood glucose level)/(pre-drug blood glucose level).

Statistical Analysis The data are expressed as mean± S.E. The statistical significance of the differences were assessed with the two-tailed Student's t-test. A p-value less than or equal to 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

From screening of the hypoglycemic effects in STZ-induced diabetic mice, amongst the bicyclic derivatives, only compounds **2** and **7** were desirable. Thus, compounds **1**, **2** and **7** began to express statistically significant differences 60 min after injection, we therefore used the 60 min data for evaluation of the effect. The other compounds did not have an effect. Compound **5** (40 mg/kg) caused strychnine-like tonic convulsion and death, and was removed from our analysis.

Compounds **1**, **2** and **7** were examined further with a glucose tolerance test. (−)-Multiflorine (**1**) had a hypoglycemic effect not only in STZ-induced diabetic mice but also in normal mice. Intraperitoneal injection of (−)-multiflorine in mice (n=10) at doses of 20 and 40 mg/kg, resulted in a marked and dose-dependent reduction in blood glucose levels (Fig. 3).

Figures 4 and 5 show the time course of the increment ratios of blood glucose levels for the synthetic compounds **2** (3 and 10 mg/kg suspended in CMC) and **7** (40 mg/kg in saline) in mice, respectively. The hypoglycemic effect of compound **2** was approximately 4 times stronger than that of (−)-multiflorine, as evaluated by glucose tolerance test in normal

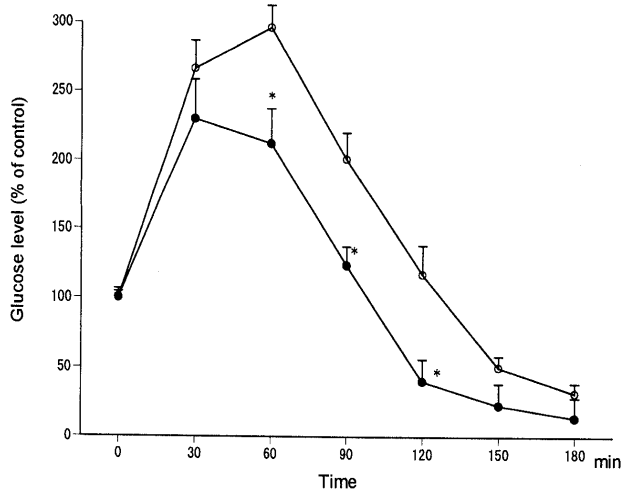

Fig. 5. Effect of Compound **7** (●; 40 mg/kg, i.p.) on the Blood Glucose Level of Normal Mice in the Oral Glucose Tolerance Test

Each value represents the mean with S.E. ($n=10$). $*p<0.05$ *vs.* vehicle (saline; ○) treated group.

mice, whereas the effect of compound **7** was weaker than that of (−)-multiflorine.

In comparing the epimer (**2** and **3**), only **2** had a significant effect. Thus, it appeared that although both stereochemistry and the position of the side chain influence the hypoglycemic potency of quinolizidin-2-one ring systems, stereochemistry in particular seems important for inducing a hypoglycemic effect. The basic structure responsible for the hypoglycemic activity was presumed to be quinolizidin-2-one, which has a substituent stereochemically similar to (−)-multiflorine (**1**) on the B-ring.

The mechanism of the hypoglycemic effect of the quinolizidin-2-one ring system was not investigated in this study. Thus, since the quinolizidin-2-one ring systems are not similar in structure to conventional drugs used in the treatment of diabetes, these ring systems may have therapeutic potential as a new type diabetes drug, not only for NIDDM, but also for IDDM. Modifications to improve potency, modify the administration formulation, and to derive detailed information on structure-activity relationships of the quinolizidin-2-one ring are currently under investigation in our laboratory.

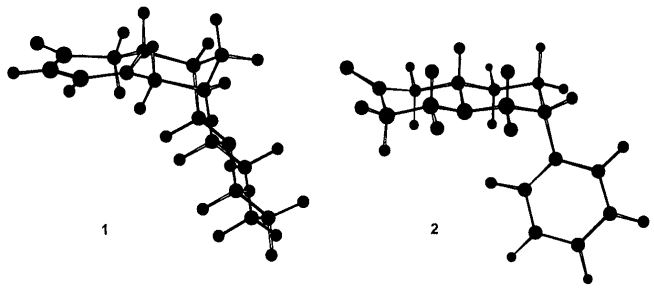

Fig. 6. Stereochemistry of (−)-Multiflorine (**1**) and Compound **2**

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