Hypoglycemic Effects of Multiflorine Derivatives in Normal Mice

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(−)-Multiflorine isolated from leguminous plants produces 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 hypoglycemic effect, several compounds bearing the quinolizidin-2-one ring system were synthesized, and their hypoglycemic effects were examined. In addition, tricyclic compounds bearing 4-pyridone were synthesized, and their hypoglycemic effects were examined. The hypoglycemic effect of a 4-pyridone-type compound was similar to that of (−)-multiflorine as measured by oral glucose tolerance testing in normal mice. No hypoglycemic effect of a 4-piperidone-type compound was observed. These results indicate that compounds possessing double bond(s) in the A ring of multiflorine may be lead compounds for a new type of diabetes drug.

Key words quinolizidin-2-one; multiflorine; hypoglycemic effect; lupine alkaloid; pyridone

Of the hypoglycemic agents that have been used clinically, biguanides and sulfonylureas have pharmacologic activity with respect to noninsulin-dependent diabetes mellitus (NIDDM), but they are ineffective in the treatment of 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. Efficient oral drugs for the treatment of both types of diabetes would therefore be useful. (−)-Multiflorine, which is known to have hypoglycemic activity when administered to mice with streptozotocin-induced diabetes, is a promising candidate. (−)-Multiflorine has been isolated from Lupinus hirsutus and Lupinus termis (Leguminosae). In Greece, L. hirsutus is used as a traditional drug for the treatment of diabetes. In Egypt, the seeds of L. termis are a popular food after the alkaloids have been removed by water immersion for several days.

Our study focused on the identification of noninsulin-dependent hypoglycemic agents derived from (−)-multiflorine isolated from L. termis. Because we suspected that the hypoglycemic effect is due to the A–B ring system of (−)-multiflorine, we synthesized 5,6-dehydromultiflorine (compound 2) and dihydromultiflorine (compound 3) (Fig. 1). The hypoglycemic effects of (−)-multiflorine and its derivatives were evaluated in oral glucose tolerance tests in normal mice.

The hypoglycemic effect of compound 2 was found to be similar to that of (−)-multiflorine in normal mice, whereas the effect of compound 3 was weaker than that of (−)-multiflorine (see Results). To define the hypoglycemic effect of the D ring of multiflorine, N-substituted tricyclic compounds bearing 4-pyridone (compounds 4–8) were synthesized (Fig. 2) and examined for hypoglycemic effects.

MATERIALS AND METHODS

Plant Material and Isolation of Lupine Aalkaloids

Seeds of L. termis (1.0 kg) were purchased in Egypt in 1998. Dry L. termis seeds were extracted three times with 75% MeOH at room temperature. The aqueous concentrate was acidified to pH 3.0 with 10% HCl, and the resulting precipitate was filtered out. The filtrate was extracted three times with CH2Cl2. The aqueous layer was made strongly alkaline with K2CO3 and extracted three times with CH2Cl2. The CH2Cl2 layers were combined, dried over Na2SO4, and concentrated in vacuo, yielding a crude base (yield, 18.2 g, 1.8%). The crude base was separated by chromatography on a silica gel with a solvent gradient of CH2Cl2:MeOH:25% NH4OH. The eluted volume of each fraction was 100 ml.

Purification of (−)-Multiflorine

The crude alkaloid mixture was separated on silica gel column chromatography and purified by preparative HPLC, resulting in purified (−)-multiflorine.

(−)-Multiflorine (I): Colorless needles, mp 108°C. [α]D −271.1° (c=0.381, MeOH). 1H-NMR (CDCl3) δ: 6.84 (1H, d, J=7.7 Hz), 4.96 (1H, d, J=7.7 Hz), 3.46 (1H, dd, 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, dd, J=12.9, 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).

13C-NMR (CDCl3) 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), 2940, 2850 (Bohlmann bands), 1630 (conjugated C=O), 1580 (conjugated –HC=CH–). EI-MS m/z: 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).

Dehydrogenation of (−)-Multiflorine

A 1.6 m n-BuLi solution in hexane was added to diisopropylamine (45 mg, 0.44 mmol) in anhydrous tetrahydrofuran (THF) (5 ml) at −78°C under nitrogen to prepare a lithium diisopropylamide solution. To this solution, (−)-multiflorine (100 mg, 0.40 mmol) in THF (3 ml) was added over 30 min and was stirred at −78°C for 1 h. Phenylselenyl chloride (92 mg, 0.48 mmol) in THF (3 ml) was then added, and the solution was stirred at this temperature for 8 h. The reaction was terminated with water, and the reaction mixture was warmed to room temperature and extracted three times with CH2Cl2. The CH2Cl2 lay-
ers were dried over Na2SO4, and the solvent was removed in vacuo, resulting in crude 5-phenylselenylmultiflorine. Crude 5-phenylselenylmultiflorine was dissolved in anhydrous CH2Cl2 (3 ml) and cooled to −60 °C. To this solution, m-chloroperbenzoic acid (150 mg, 0.66 mmol) in anhydrous CH2Cl2 (3 ml) was then added, and the solution was stirred for 6 h at −60 °C and warmed to room temperature. Water was added to the reaction mixture, and the CHCl3 layer was separated out. The aqueous layer was extracted three times with CHCl3. The combined CHCl3 layers were dried over Na2SO4, and the solvent was removed in vacuo. The residue was purified on silica gel column chromatography (solvent system CH2Cl2 : MeOH : 28% NH4OH = 90 : 9 : 1) to give multiflorine as a single isomer (pale yellow oil; yield, 93 mg, 93%).

The reaction mixture was allowed to come to room temperature and was then stirred for 3 h. The reaction was terminated with water, and the solution was extracted three times with CH2Cl2. The organic layers were combined, dried over Na2SO4, and the organic solution was removed in vacuo. The residue was purified with silica gel column chromatography (yield, 756 mg, 82%).

Stille Coupling, Bipyridyl (9) 4-Methoxy-2-tritylstannanlypyridine (1420 mg, 3.57 mmol), ethyl 5-bromonicotinate (1642 mg, 7.14 mmol), triphenylphosphine (93 mg, 0.357 mmol), and tetrais(triphenylphosphine)palladium(0) (206 mg, 0.179 mmol) were dissolved in p-xylene (30 ml) and refluxed under nitrogen for 10 h. After cooling, water was added to the reaction mixture, and the solution made alkaline with NaHCO3 solution. The xylene layer was removed, and the aqueous solution was extracted twice with CH2Cl2. The organic layers were combined, dried over Na2SO4, and the organic solution was removed in vacuo. The residue was purified with silica gel column chromatography (yield, 114 mg, 93%).

Hydrogenation of (−)-Multiflorine (−)-Multiflorine (100 mg, 0.40 mmol) was dissolved in AcOEt (10 ml) and hydrogenated with PtO2 (10 mg) as a catalyst. The catalyst was removed by filtration, and the solvent was removed in vacuo. The resulting residue was purified on silica gel column chromatography (solvent system CH2Cl2 : MeOH : 28% NH4OH = 90 : 9 : 1) to give 4-hydroxysparteine as a single isomer (pale yellow oil; yield, 93 mg, 93%).

1H-NMR (CDCl3) δ: 7.19 (1H, d, J = 7.7 Hz), 6.39 (1H, dd, J = 7.7, 2.8 Hz), 6.19 (1H, d, J = 2.8 Hz), 4.12 (1H, dd, J = 12.7, 6.3 Hz), 3.92 (1H, d, J = 12.7 Hz), 3.35 (1H, d, J = 2.8 Hz), 2.93 (1H, d, J = 11.8 Hz), 2.90 (1H, m), 2.76 (1H, dd, J = 13.8, 2.8 Hz), 2.68 (1H, ddd, J = 13.8, 1.9, 1.9 Hz), 2.49 (1H, d, J = 8.3 Hz), 2.05 (1H, m), 2.01 (6H, m), 1.12 (2H, m).

13C-NMR (CDCl3) δ: 178.9 (s), 153.5 (s), 139.7 (d), 117.9 (d), 116.0 (d), 57.4 (t), 54.3 (t), 52.0 (t), 34.8 (t), 32.6 (d), 25.4 (t), 22.1 (t), 21.0 (t), 18.8 (t). IR (film) cm−1: 3410, 2930, 2860, 1650, 1550, 1360, 1200, 1200, 1140. HR-ESI-MS m/z: Found 248.1575 (Calcd for C15H24N2O: 248.1889). EI-MS m/z: 248 (100), 162 (59), 136 (21).
Cyclization of 11, N-Benzyl Tricyclic Pyridine (4)

The alcohol 11 (100 mg, 0.32 mmol) was dissolved in a 48% HBr solution (5 ml). The solution was refluxed for 12 h. After cooling, the reaction mixture was made alkaline with 20% NaOH and extracted three times with CHCl3. The CHCl3 layers were combined, dried over Na2SO4, and the solvent was removed in vacuo to give a methyl-1,2,3,4,5,6-hexahydro-1,5-methano-pyrido[1,2-a][1,5]diazocin-10 (4).

Deprotection of Compound 4, Secondary Amine 5

Compound 4 (150 mg, 0.53 mmol) in EtOH (10 ml) was hydrogenated over 10% palladium on carbon for 3 h. The catalyst was removed by filtration, and the filtrate was evaporated in vacuo. The residue was purified on silica gel column chromatography (CH2Cl2: MeOH = 28% NH4OH = 90: 9: 1) to yield compound 5 as a colorless oil (yield, 45%, 45%).

N-Methylation, N-Methyl Tricyclic Compound (6)

Secondary amine 5 (56 mg, 0.29 mmol) in EtOH (5 ml) was treated with HCHO (17.4 mg, 0.5 mmol) and NaBH4CN (18.2 mg, 0.29 mmol) for 10 h. The solution was made acidic by the addition of 5% HCl solution, and the solution was stirred until there was no further generation of gas. After cooling, the solution was made alkaline with K2CO3 and extracted with CHCl3 three times. The CHCl3 layers were combined, dried over Na2SO4, and the solvent was removed in vacuo. The residue was purified on silica gel column chromatography (CH2Cl2: MeOH = 9: 1) to yield compound 6 as a colorless oil (yield, 40 mg, 66%).

13C-NMR (CDCl3) δ: 7.25 (1H, d, J = 7.6 Hz), 6.40 (1H, dd, J = 7.6, 2.8 Hz), 6.25 (1H, d, J = 2.8 Hz), 4.11 (1H, dd, J = 12.5, 6.2 Hz), 3.92 (1H, d, J = 12.5 Hz), 2.88 (3H, m), 3.25 (1H, m), 2.27 (1H, m), 2.22 (1H, m), 2.15 (3H, s), 1.94 (1H, dm, J = 13.5 Hz), 1.78 (1H, dm, J = 13.5 Hz). 13C-NMR (CDCl3) δ: 178.8 (s), 153.2 (s), 140.0 (d), 117.7 (d), 116.0 (d), 116.1 (t), 61.5 (t), 55.7 (t), 46.1 (q), 34.6 (d), 27.9 (d), 25.5 (t). HR-ESI-MS m/z: Found 204.1274 (Calcd for C13H16N2O: 204.1262). MS m/z: 204 (M+, 72), 84 (39), 58 (100), 49 (52).

N-Allyl Tricyclic Compound (7)

Secondary amine 5 (56 mg, 0.29 mmol) in dimethyl formamide (5 ml) was treated with allyl bromide (70 mg, 0.58 mmol) and K2CO3 (120 mg, 0.87 mmol) at 50 °C for 2 h. Inorganic compounds were removed by filtration, and the filtrate was evaporated in vacuo. The residue was purified by silica gel column chromatography (CH2Cl2: MeOH = 9: 1) to yield an N-allyl compound (7) as a pale yellow oil (yield, 50 mg, 74%).

1H-NMR (CDCl3) δ: 7.31 (1H, d, J = 7.4 Hz), 6.53 (1H, dd, J = 7.4, 2.6 Hz), 6.36 (1H, d, J = 2.6 Hz), 5.60 (1H, m), 5.07 (1H, dm, J = 9.0 Hz), 5.04 (1H, dm, J = 18.2 Hz), 4.16 (1H, dd, J = 12.9, 6.2 Hz), 3.98 (1H, d, J = 12.9 Hz), 2.88—2.99 (5H, m), 2.2—2.4 (3H, m), 1.96 (1H, dm, J = 12.8 Hz). HR-ESI-MS m/z: Found 230.1431 (Calcd for C12H20N3O2: 230.1419).

N-Propyl Tricyclic Compound (8)

N-Allyl tricyclic compound 7 (30 mg, 0.13 mmol) in EtOH (5 ml) was hydrogenated over 10% palladium on carbon (8 mg) under ambient pressure. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo. The residue was purified on silica gel column chromatography (CH2Cl2: MeOH = 9: 1) to yield N-propyl tricyclic compounds as a pale yellow oil (yield, 25 mg, 82%).

Animals

Six-weeks-old male ICR mice (Tokyo Laboratory Animal Science Co., Ltd., Tokyo, Japan), weighing approximately 30 g, 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. Mice were fasted for 12 h prior to oral glucose tolerance testing. Studies were carried out in accordance with the Declaration of Helsinki and/or 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 Japanese Ministry of Education, Culture, Sports, Science and Technology.

The hypoglycemic effect of the compounds were evaluated in a glucose tolerance test. Each mouse was injected intraperitoneally with the test drug, followed by oral administration of 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 injection. The increment ratios of blood glucose levels were calculated for each animal according to the following formula: 100×(postdrug blood glucose level−predrug blood glucose level)/(predrug blood glucose level).

Statistical Analysis

Data are expressed as mean±S.E. The statistical significance of differences was assessed using the Bonferroni-Dunn test. p<0.05 was considered statistically significant.
RESULTS AND DISCUSSION

It has been reported that 5,6-dehydromultiflorine can be isolated from *L. termis*, but we were unable to do so. Therefore, multiflorine (1) was first transformed to 5-phenylselenylmultiflorine and then treated with m-chloroperbenzoic acid, yielding 5,6-dehydromultiflorine (2) as a 4-pyridone-type alkaloid (Fig. 1). Dihydromultiflorine (3) was not isolated from *L. termis*, but was isolated from genus *Lupinus* plants. This compound was also transformed from (-)-multiflorine (1) with the following method. (-)-Multiflorine was treated with platinum oxide-catalyzed hydrogenation to create 4-hydroxysparteine, and the hydroxy group was then oxidized to yield a 4-piperidone-type alkaloid, dihydromultiflorine (3) (Fig. 1).

The syntheses of tricyclic compounds were similar to that of cytisine (Fig. 2). 4-Methoxy-2-tributylstannanylpyridine and ethyl 5-bromonicotinate were selected as starting materials. Compound 9 was synthesized by Stille coupling. The ester was reduced with LiAlH₄ to give a dipyridyl alcohol (10). Selective N-benzylation of the nitrogen arising from nicotinate was performed to give a pyridinium salt. The pyridinium salt was hydrogenated over platinum oxide to give 3,5-cis piperidine (11) as a single isomer by NMR. Mesylation of the primary alcohol (11) followed by heating yielded benzyl-protected tricyclic 4-pyridone (4). Hydrogenolysis of compound 4 with a palladium catalyst gave a secondary amine (5). The secondary amine group in compound 5 was alkylated with methyl iodine and allyl bromide to yield compounds 6 and 7, respectively. Reduction of the double bond in the allyl group of compound 7 reduced with palladium gave an N-propyl amine (8).

Compounds 1—8 were analyzed in glucose tolerance tests. The time courses of the increment ratios of blood glucose levels for tetracyclic compounds 1, 2, and 3 (30 mg/kg in saline) and tricyclic compounds 4—8 are shown in Figs. 3 and 4, respectively. We selected the dose 30 mg/kg because (-)-multiflorine (1) began to show a statistically significant effect from vehicle at that dose.

Among the tetracyclic compounds tested, only compounds 1 and 2 had significant hypoglycemic effects. The chemical structures of these compounds are similar to each other. Thus it appears that double bond(s) in the A–B ring system of multiflorine influence hypoglycemic potency. Among the tricyclic compounds tested, compounds 5 and 6 had significant hypoglycemic effects, similar to that of multiflorine. We suspect that the D ring in (-)-multiflorine (1) is not necessary for the hypoglycemic effect and that small substituted groups such as a methyl group influence the potency of the hypoglycemic effect.

Cytisine is a common component in leguminous plants and it behaves as a partial agonist of neuronal nicotinic receptors. However, because of the toxicity of 2-pyridone-type lupine alkaloids, cytisine is not used therapeutically. The administration of (-)-cytisine (30 mg/kg, intraperitoneally)
induced convulsive seizures in mice, and almost all died. Acute toxicity of multiflorine or 5,6-dehydromultiflorine (2) as well as compounds 5 and 6 was not found in this study. Thus the position of the oxo-group in the A–B ring system of multiflorine has hypoglycemic effects with no acute toxicity.

The mechanism of the hypoglycemic effect of the quinolizidin-2-one ring system was not investigated in this study. The quinolizidin-2-one ring system differs in structure from conventional drugs used for the treatment of diabetes and may have therapeutic potential as a new type of diabetes drug. Modifications to improve the potency and administration as well as a detailed study of structure–activity relations of the quinolizidin-2-one ring system are currently under way in our laboratory.

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