Two New 2-Arylbenzofuran Derivatives from Hypoglycemic Activity-Bearing Fractions of *Morus insignis*

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Ethyl acetate- and *n*-butanol-soluble fractions of the leaves of *Morus insignis* showed a significant hypoglycemic activity on streptozotocin (STZ)-induced hyperglycemic rats. From these hypoglycemic activity-showing fractions, two new compounds, mulberrofuran U (2) and moracin M-3′-*O*-*β*-D-glucopyranoside (3) were isolated, along with six known compounds [*β*-sitosterol, *β*-sitosterol-3-*O*-*β*-glucopyranoside, ursolic acid, moracin M (1), kaempferol-3-*O*-*β*-glucopyranoside and quercetin-3-*O*-*β*-glucopyranoside] and the structures of the new compounds were determined by spectroscopic and chemical methods.

Keywords *Morus insignis*; Moraceae; mulberry; mulberrofuran U; moracin M-3′-*O*-*β*-D-glucopyranoside; hypoglycemic activity

Mulberry (*Morus*) plants are widely cultivated in China, Japan and other countries for their leaves, which are used to feed silk worms, while the root bark of mulberry is a traditional medicine used as an antiphlogistic, diuretic, expectorant[1] and laxative. A number of complex phenolic compounds, isolated from mulberry root bark have been reported to have a significant hypotensive effect,[2-4] as well as antifungal[5,6] and antibacterial[7] activities. It has also been reported that a glycoprotein isolated from *Morus alba* showed a strong hypoglycemic activity.[8] The alcoholic extract of leaves of *M. insignis* BUR. effectively lowered the blood glucose level in streptozotocin (STZ)-induced diabetic rats. Hence the hypoglycemic activity-guided fractionation of *M. insignis* BUR. was carried out and two new compounds were isolated, along with six known compounds, from the hypoglycemic activity-showing fractions of the leaves. In the present paper, we wish to report the hypoglycemic acitivity of various fractions of the aqueous alcoholic extract of *M. insignis* and the structure elucidation of the two new compounds, mulberrofuran U (**2**) and moracin M-3′-*O*-*β*-D-glucopyranoside (**3**), based on spectroscopic and chemical evidence.

During preliminary screening tests for hypoglycemic activity, the aqueous alcoholic extract of the leaves of *M. insignis* collected from Argentina was found to be more effective to lower blood glucose level in STZ-induced diabetic rats than extract from other species of mulberry collected from various places in Japan, so that this plant was further taken for investigation. Studies were made in normal as well as STZ-induced diabetic rats by long term treatment (i.p.) and single-dose treatment (i.p. and *p.o.*) with the various fractions of the alcoholic extract of this plant, and an attempt was made to isolate the active principle or principles.

Twenty doses of 100 mg/kg (i.p., twice a day) of 70% EtOH extract reduced the blood glucose level of STZ-induced diabetic rats by 34.5% from the control level (Fig. 1). In this experiment blood glucose level was checked after injecting 5 doses (i.p.) and blood samples were collected 6 h after administration of the last dose. The results (Fig. 1) suggested that the aqueous alcoholic extract contains hypoglycemic principles.

By observing the hypoglycemic activity, the aqueous alcoholic extract was fractionated into EtOAc-, *n*-BuOH- and water-soluble fractions (see Experimental) and the blood glucose-lowering effect of these fractions was studied in normal rats. A single dose of 50 mg/kg (i.p.) of each of these fractions was administered, and blood glucose level was checked 7 and 24 h later. The EtOAc- and *n*-BuOH-soluble fractions were found to lower the blood glucose level significantly within 7 h, while at 24 h the blood glucose level had returned to normal (Table I). The water-soluble fraction did not show any significant activity even when 5 doses (i.p.) of 100 mg/kg were administered.

Again, the hypoglycemic activity of the EtOAc-soluble fraction, *n*-BuOH-soluble fraction and aqueous alcoholic extract was examined in STZ-induced diabetic rats by administering a single dose of 100 mg/kg (*p.o.*) and blood glucose level was observed at 3 and 6 h after the drug administration. In this experiment also the *n*-BuOH- and EtOAc-soluble fractions both showed a significant hypoglycemic activity (Table II), though the *n*-BuOH-soluble

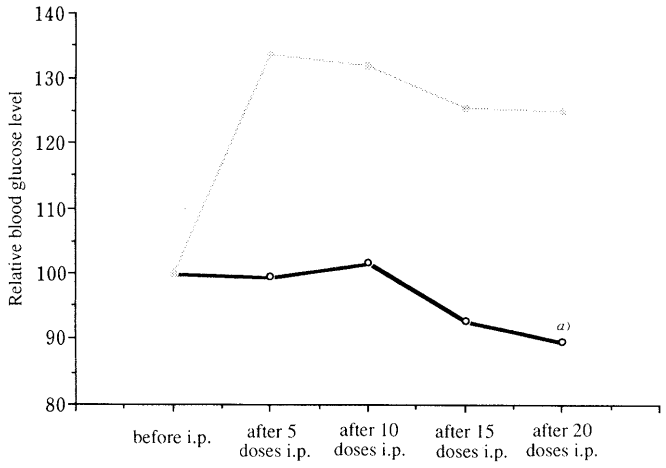


Fig. 1. Effects of Aqueous Ethanolic Extract of *Morus insignis* on the Blood Glucose Level in STZ Induced Diabetic Rats

▒▒▒, control; —○—, 1. 1: Aqueous ethanolic extract of *M. insignis*; dose 100 mg/kg/dose (twice a day). Blood glucose level after STZ administration (before i.p.) ranged from 300 to 450 mg/dl and adjusted to 100. Results to means of five experiments ($n=5$). Significantly different from control value, *a*) $p<0.05$.

TABLE I. Effects of Different Fractions of *Morus insignis* (Single-Dose Administration, i.p.) on Blood Glucose Level in Normal Rats

Group	Dose (mg/kg)	Glucose level[b] (decrease %)[c]					
		0 h[a]	7 h		24 h		
I	—	153.8±4.2	162.3±3.1		154.3± 1.7		
		(100.0±2.7)	(105.5±2.0)		(100.3± 1.1)		
II	50	147.6±3.2	142.2±6.2[d]	(8.7)	149.4±10.3	(−0.9)	
		(100.0±2.2)	(96.3±4.2)		(101.2± 7.0)		
III	50	148.2±3.7	137.2±6.1[d]	(12.2)	151.2± 6.1	(−1.7)	
		(100.0±2.5)	(92.6±4.1)		(102.0± 4.1)		
IV	50	152.0±3.1	155.3±3.3	(3.1)	153.3± 3.3	(−0.5)	
		(100.0±2.0)	(102.2±2.2)		(100.8± 2.2)		

I: Control (physiological saline treated). II: EtOAc-soluble fraction treated. III: *n*-BuOH-soluble fraction treated. IV: Water soluble fraction treated. *a*) Glucose level before administering the drugs or saline. *b*) Glucose level expressed in mg/dl. *c*) Decrease in blood glucose level relative to before i.p. drug injection, and expressed as % compared with the control. Results are mean ± S.E. of five experiments ($n=5$). Significantly different from control value, *d*) $p<0.05$.

TABLE II. Effects of Different Fractions of *Morus insignis* (Single-Dose Administration, *p.o.*) on Blood Glucose Level STZ Induced Diabetic Rats

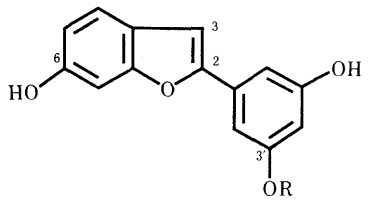
Group	Dose (mg/kg)	Glucose level[b] (decrease %)[c]					
		0 h[a]	3 h		6 h		
I	—	445±20	521±30		481±35		
		(100.0±4.5)	(117.1±6.7)		(108.1±7.9)		
II	100	439±20	478±31	(7.0)	457±32	(3.7)	
		(100.0±4.5)	(108.9±7.1)		(104.1±7.2)		
III	100	438±20	460±32[d]	(10.3)	469±36	(0.9)	
		(100.0±4.6)	(105.0±7.3)		(107.1±8.2)		
IV	100	440±18	393±20[d]	(23.7)	419±33	(11.9)	
		(100.0±4.1)	(89.3±4.5)		(95.2±7.5)		

I: Control (physiological saline treated). II: 70% EtOH extract treated. III: EtOAc soluble fraction treated. IV: *n*-BuOH soluble fraction treated. *a*) Glucose level before administering the drugs or saline. *b*) Glucose level expressed in mg/dl. *c*) Decrease in blood glucose level relative to before *p.o.* dosing expressed as % *vs.* the control. Results are mean ± S.E. of six experiments ($n=6$). Significantly different from control value, *d*) $p<0.01$.

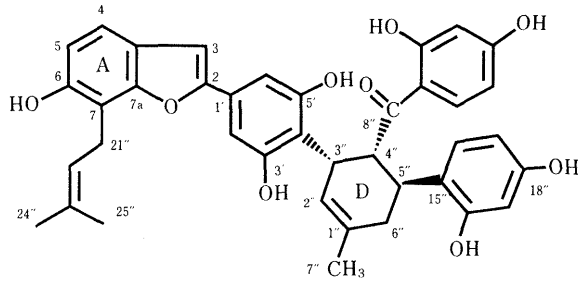
fraction lowered the blood glucose level more effectively.

This is the first report that hypoglycemic activity of leaves of *M. insignis* was observed in normal and STZ-induced diabetic rats. Analysis of active fractions led to the isolation of two new compounds along with six known compounds. The structures of these compounds were elucidated based on the spectroscopic and chemical evidence.

Compound **1** was isolated from the EtOAc-soluble fraction (see Experimental), mp 275 °C (MeOH) as a light pink crystalline solid. It showed the molecular ion peak (M^+) at m/z 242 in the electron impact mass spectrum (EI-MS), and high-resolution MS indicated the molecular formula to be $C_{14}H_{10}O_4$. The UV absorptions at λ_{max} 214 (9837), 304 (10125) (sh), and 318 (11365) nm were indicative of an arylbenzofuran-type compound.[9] Its IR spectrum suggested that **1** was a polyphenol-type compound without a carbonyl function. It showed 12 carbon signals in the aromatic region in its ^{13}C-NMR spectrum, 6 signals due to singlet (s) carbons and 6 due to aromatic methine carbons (d) according to the distortionless enhancement by polarization transfer (DEPT) spectrum. Among the 6 singlet signals, 4 were observed at low field (δ_C 160.3, 157.8, 157.1, 156.7) due to aromatic carbon bonding with oxygen, while two were observed at relatively high field (δ_C 134.4, 123.7). The signals at δ_C 160.3 and 104.8 were twice as intense as other relative carbon signals, suggesting that these signals were due to a set of two equivalent carbons. The ^{1}H-NMR spectrum showed the signals due to 7 aromatic protons. A set of three protons coupling with one another at δ_H 7.33 (1H, d, $J=8.5$ Hz), 6.91 (1H, d, $J=2.5$ Hz), and 6.73 (1H, dd, $J=8.5$, 2.5 Hz) in one ring, another set of three protons coupling with one another at δ_H 6.77 (2H, d, $J=2.5$ Hz) and 6.26 (1H, t, $J=2.5$ Hz) in another ring, and a singlet signal at δ_H 6.88 (1H, s) in another ring were observed. Based on the nature of the compounds previously isolated from *Morus* plants and data obtained for **1**, we suggested the tentative structure of moracin M (**1**). The structure was confirmed by ^{1}H–^{1}H shift correlation spectroscopy (COSY),



1: R=H, **3**: R=β-D-glucopyranosyl



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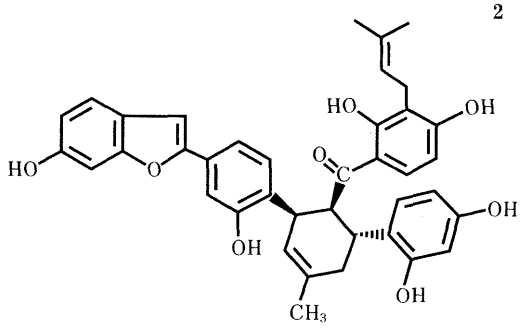

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Chart 1

TABLE III. ^{1}H-NMR (400 MHz) Spectral Data for Compounds **1**—**3**

Proton	**1**[a,c]	**2**[b,c]	Proton	**3**[b,d]
3	6.88, s	6.93, s	3	7.18, s
4	7.33, d, (8.5)	8.06, d, (9.0)	4	7.40, d, (9.0)
5	6.73, dd, (8.5, 2.5)	6.38, d, (9.0)	5	6.75, dd, (9.0, 2.0)
7	6.91, d, (2.5)		7	6.95, br d, (2.0)
2′	6.77, d, (2.5)	6.59, s	2′	6.89, br s
4′	6.26, t, (2.5)	—	4′	6.45, t, (2.0)
6′	6.77, d, (2.5)	6.59, s	6′	6.98, br s
2″	—	5.51, br d, (1.5)	Sugar	
3″	—	4.13, br	1	5.86, d, (7.5)
4″	—	4.43, dd, (7.5, 6.5)	2	3.25, m
5″	—	3.83, td, (6.5, 6.0)	3	3.30, m
6″-H_a	—	2.35, br d, (17.0)	4	3.20, m
6″-H_b	—	2.07, br d, (17.0)	5	3.35, m
7″	—	1.80, br s	6-H_a	3.74, dd, (12.0, 4.0)
11″	—	6.89, d, (2.5)	6-H_b	3.50, dd, (12.0, 4.0)
13″	—	6.72, dd, (8.5, 2.5)	2-OH	5.34, d, (5.0)
14″	—	7.36, d, (8.5)	3-OH	5.12, d, (3.5)
17″	—	6.28, d, (2.5)	4-OH	5.05, d, (5.0)
19″	—	6.08, dd, (8.5, 2.5)	6-OH	4.54, d, (4.5)
20″	—	6.77 d, (8.5)		
21″	—	3.13, d, (7.0)		
22″	—	5.08, tt, (7.0, 1.2)		
24″	—	1.06, br s		
25″	—	1.55, br s		

Chemical shift in δ ppm, coupling constant (J) expressed in Hz in parenthesis and measured in the solvent *a*) CD_3OD and *b*) DMSO-d_6, taking TMS as an internal standard. Assignments were supported by *c*) ^{1}H–^{1}H COSY *d*) ^{1}H–^{1}H COSY and NOE measurements.

TABLE IV. ^{13}C-NMR (100 MHz) Spectral Data for Compounds **1**—**3**

Carbon	**1**[a,c]	**2**[b,c]	Carbon	**3**[b,c]
2	156.7 (s)	155.2 (s)	2	153.6 (s)
3	102.8 (d)	102.7 (d)	3	102.2 (d)
3a	123.7 (s)	120.9 (s)	3a	120.8 (s)
4	122.5 (d)	121.5 (d)	4	121.3 (d)
5	113.8 (d)	112.3 (d)	5	112.6 (d)
6	157.1 (s)	162.5 (s)	6	155.9 (s)
7	99.3 (d)	113.9 (s)	7	97.5 (d)
7a	157.8 (s)	162.3 (s)	7a	155.4 (s)
1′	134.4 (s)	130.4 (s)	1′	131.7 (s)
2′	104.8 (d)	102.6 (d)	2′	103.7 (d)
3′	160.3 (s)	156.2 (s)	3′	159.1 (s)
4′	104.2 (d)	112.5 (s)	4′	103.3 (d)
5′	160.3 (s)	156.2 (s)	5′	158.7 (s)
6′	104.8 (d)	102.5 (d)	6′	104.7 (d)
1″	—	132.0 (s)	Sugar	
2″	—	122.8 (d)	1	100.7 (d)
3″	—	32.8 (d)	2	73.3 (d)
4″	—	46.6 (d)	3	77.4 (d)
5″	—	33.8 (d)	4	69.8 (d)
6″	—	33.9 (d)	5	76.7 (d)
7″	—	23.4 (q)	6	60.2 (t)
8″	—	207.4 (s)		
9″	—	115.0 (s)		
10″	—	156.9 (s)		
11″	—	101.4 (d)		
12″	—	156.9 (s)		
13″	—	105.9 (d)		
14″	—	132.0 (d)		
15″	—	122.4 (s)		
16″	—	155.5 (s)		
17″	—	102.6 (d)		
18″	—	156.6 (s)		
19″	—	105.9 (d)		
20″	—	127.5 (d)		
21″	—	21.2 (t)		
22″	—	122.4 (d)		
23″	—	130.2 (s)		
24″	—	17.6 (q)		
25″	—	25.4 (q)		

Chemical shifts in δ ppm, measured in *a*) CD_3OD *b*) DMSO-d_6 where the multiplicities of carbon signals were determined by means of the DEPT method, and indicated as s, d, t, and q. *c*) ^{1}H–^{13}C COSY, ^{1}H–^{13}C long range COSY and HMBC spectra were measured.

^{1}H–^{13}C COSY, and ^{1}H–^{13}C long-range COSY experiments. Literature survey showed that complete NMR spectral data for **1** have not been reported, but a number of derivatives of **1** have been described.[4,10] The ^{1}H- and ^{13}C-NMR data of known derivatives of **1**[4,10] and ^{1}H-NMR and other physical data (lit. mp 260—262 °C, quite different from our observation) of moracin M (6,3′,5′-trihydroxy-2-phenylbenzofuran)[11] were also compared with the data shown in Tables III and IV. This compound was concluded to be moracin M, previously isolated from *M. laevigara*.[11] The 2-arylbenzofuran moiety of **1** is also present in **2** and **3**.

Compound **2** (mulberrofuran U) isolated from the EtOAc-soluble fraction (see Experimental) was a light yellow amorphous powder, soluble in EtOAc, acetone and MeOH. It gave a violet fluorescence in UV light and a positive $FeCl_3$ test. It was observed as a single spot on a normal silica gel-G coated TLC plate with the solvent systems MeOH : $CHCl_3$ (1 : 9) (Rf = 0.5), acetone : hexane (4 : 6) (Rf = 0.3) and acetone : benzene (1 : 19) (Rf = 0.2). It decomposed at 286 °C and was optically active, $[\alpha]_D$ +128° (MeOH, c = 0.13). The UV absorptions at λ_{max} 207 (40355) and 318 (28576) nm showed the presence of an arylbenzofuran moiety. The IR absorptions at 3400, 1625, and 1505 cm^{-1} suggested a phenolic compound containing a conjugated carbonyl group. The positive ion fast atom bombardment mass spectrum (FAB-MS) showed peaks at m/z 649 $(M+H)^+$ and 671 $(M+Na)^+$ and negative ion FAB-MS showed a peak at m/z 647 $(M-H)^-$, suggesting the molecular weight to be 648, and the composition for the peak at m/z 671 was calculated as $C_{39}H_{36}O_9Na$. So based on the high-resolution MS, the molecular formula was assigned as $C_{39}H_{36}O_9$. The complete ^{13}C-NMR and DEPT spectra indicated the presence of 39 carbons: 26 aromatic carbons (CH × 11, C × 6, C–O × 9), 12 others (CH_3 × 3, CH_2 × 2, CH × 3, >C=CH × 2) and one ketonic carbon. A part of the ^{1}H-NMR spectrum was very similar to that of **1**, so it was suggested that **2** is a complex derivative of **1**. Eleven aromatic protons, four of them *ortho*-coupling, and two of them *ortho*- and *meta*-coupling, and two of them *meta*-coupling, as well as a singlet signal due to one proton and another singlet signal due to two protons were observed in the ^{1}H-NMR spectrum (Table III). Besides this, a tetra-substituted cyclohexene ring was indicated by the signals at δ_H 5.51 (1H, br d, J = 1.5 Hz), 4.13 (1H, br), 4.43 (1H, dd, J = 7.5, 6.5 Hz), 3.83 (1H, td, J = 6.5, 6.0 Hz), 2.35 (1H, br d, J = 17.0 Hz), and 2.07 (1H, br d, J = 17.0 Hz) in the ^{1}H-NMR spectrum and signals at δ_C 132.0 (s), 122.8 (d), 32.8 (d), 46.6 (d), 33.8 (d), 33.9 (d) in the ^{13}C-NMR spectrum. The positions of substituents in the cyclohexene ring were confirmed by examination of the ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY and ^{1}H-detected heteronuclear multiple-bond multiple-quantum coherence (HMBC) spectra.[12] Some of the important HMBC correlations are shown by the arrows in Chart 2. The NMR data showed that the structure of **2** was similar to those of Diels–Alder adduct type compounds such as mulberrofuran C,[4] chalcomoracin (**4**)[13,14] and mulberrofuran T,[15] previously isolated from

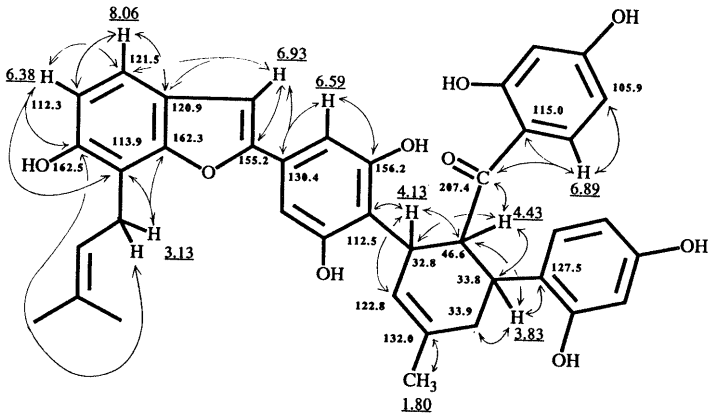

Chart 2

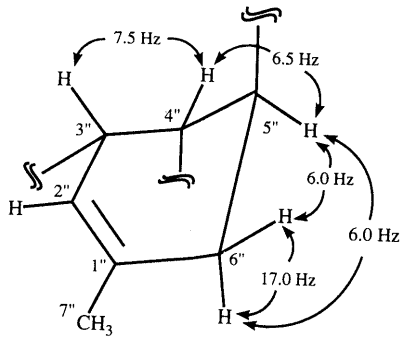

Fig. 2. Relative Configuration Proposed for Ring D of Mulberrofuran U

the root bark of *M. alba*.

At first, it was thought that **2** was chalcomoracin (**4**) but some of the physical characters such as optical rotation, melting point and some NMR signals were different. Clear differences were observed when two-dimensional NMR experiments were performed. In the ^{1}H-NMR spectrum, there was only one singlet signal due to one proton in the aromatic region at δ_H 6.93 (s), and this showed a long-range correlation with the carbon signals at δ_C 121.5 in the HMBC spectrum. This carbon (δ_C 121.5) showed a correlation with the proton at δ_H 8.06 (1H, d, J=9.0 Hz) in the ^{1}H–^{13}C COSY spectrum. The proton at δ_H 8.06 coupled with the proton at δ_H 6.38 (1H, d, J=9.0 Hz) and also showed a correlation with the carbon at δ_C 112.3 (d) in the ^{1}H–^{1}H COSY and ^{1}H–^{13}C long-range COSY spectra, respectively. Again, the proton at δ_H 6.38 (1H, d, J=9.0 Hz) showed a correlation with the carbon at δ_C 113.9 in the ^{1}H–^{13}C long-range COSY spectrum, and based on these results the carbon at δ_C 113.9 was assigned at C_7. An isoprenyl group was deduced from the signals at δ_H 3.13 (2H, d, J=7.0 Hz), 5.08 (1H, tt, J=7.0, 1.2 Hz), 1.06 (3H, br s), 1.55 (3H, br s) in the ^{1}H-NMR spectrum and at δ_C 21.2 (t), 122.4 (d), 130.2 (s), 25.4 (q), 17.6 (q) in the ^{13}C-NMR spectrum. The position for the isoprenyl group was suggested to be at C_7 based on the correlation between the $C_{21''}$ proton at δ_H 3.13 (2H, d, J=7.0 Hz), and C_7 in the HMBC spectrum. The methylene protons of $C_{21''}$ also showed correlations with C_6 and C_{7a} in the HMBC spectrum. The other important correlations are shown in Chart 2. Based on these results the structure for **2** was suggested to be as shown in Chart 2.

Based on chemical shift, ^{1}H–^{1}H COSY, coupling constant value (J), and optical rotation the relative configuration of ring D was proposed to be as shown in Fig. 2. According to a molecular model, a slightly distorted half-chair conformation of cyclohexene ring was one of the strainless conformations. Assuming the possible conformation of ring D to be a half chair conformation with $C_{5''}$ flipping up above the plane of the ring (Fig. 2), the relative configuration was suggested to be as follows according to the coupling constant (J) values. Two geminal methylene protons (H_a and H_b) at $C_{6''}$ were coupled with each other (17.0 Hz) and also with the $C_{5''}$-H proton (6.0 Hz). This value suggested $C_{5''}$-H to be equatorial. The coupling constant of $C_{5''}$-H was observed to be 6.5 Hz with $C_{4''}$-H so that $C_{4''}$-H was supposed to be equatorial. The coupling constant between $C_{4''}$-H and $C_{3''}$-H was found to be 7.5 Hz due to the minimum dihedral angle between $C_{4''}$-H and $C_{3''}$-H, since the bulky group at $C_{5''}$ was axial, so that $C_{3''}$-H was slightly pushed outside the ring, which might minimize the dihedral angle between $C_{4''}$-H and $C_{3''}$-H. In the ^{1}H–^{1}H COSY spectrum, $C_{3''}$-H showed a strong cross peak with the $C_{1''}$ methyl group, which also suggested that the protons at $C_{3''}$ and $C_{1''}$ methyl group should be almost in the same plane. Thus, the relative configuration of the bulky groups at $C_{3''}$, $C_{4''}$ and $C_{5''}$ was suggested as *cis–trans*, which was also supported by the optical rotation.[16] Compound **2** was dextrorotatory, since it showed specific rotation $[\alpha]_D$ +128°, so the configuration was assigned as *cis–trans* for ring D, and by comparing the optical rotation and J-values of mulberrofuran T[15] and other related compounds,[16] the absolute configuration was suggested to be 3″*S*,4″*R* and 5″*S*. On the basis of these findings the structure of **2** was concluded to be as shown in Chart 1. Compound **2** was named mulberrofuran U.[17]

The structure of mulberrofuran U was found to be very similar to those of mulberrofuran C, chalcomoracin (**4**) and mulberrofuran T. Mulberrofuran C has no isoprenyl group, so that mulberrofuran U is a derivative of mulberrofuran C. The position of the isoprenyl group and the configuration of ring D of mulberrofuran U and chalcomoracin (**4**) were different, so that mulberrofuran U is an isomer of chalcomoracin (**4**). There were two isoprenyl groups in mulberrofuran T at the C_7 and $C_{11''}$ positions but only one isoprenyl group was found in mulberrofuran U at C_7. Such compounds may be synthesized biogenetically[13] *via* Diels–Alder type reaction in the presence of enzymes. A number of Diels–Alder adduct-type compounds have been

reported from mulberry plants, but only a few of them contain the isoprenyl group at the C_7 position, so that mulberrofuran U is biogenetically interesting.

Compound **3**, a pale yellow crystalline solid obtained on purification of the *n*-BuOH-soluble fraction of the alcoholic extract by repeated Sephadex LH-20 column chromatography followed by recrystallization with methanol-chloroform mixture, melted at 284 °C. It gave blue fluorescence with UV-light and a brown color with alcoholic $FeCl_3$ on a silica gel-coated plate. It was levorotatory, $[\alpha]_D$ −43.1° (MeOH, $c=0.87$). The UV and IR absorption signals of **3** were quite similar to those of **1**. The FAB-MS showed the quasi-molecular ion peak at m/z 405 $(M+H)^+$ and ^{13}C-NMR and DEPT spectra showed twenty carbons; 14 aromatic carbons (CH × 7, C × 2, C–O × 5) and six sugar carbons (CH_2–O × 1, CH–O × 5). These facts supported the proposed molecular formula, $C_{20}H_{20}O_9$. The ^{1}H-NMR spectrum of **3** showed seven proton signals in the aromatic region, as did **1**, but the signals due to the ring C protons of **3** were found to be slightly different from those of **1** (Table III). Thus, **3** was suggested to be a glycoside of **1**. The ^{1}H- and ^{13}C-NMR spectra suggested that the $C_{2'}$ and $C_{6'}$ as well as $C_{3'}$ and $C_{5'}$ positions of **3** were not equivalent, in contrast to **1**, and hence the six-membered sugar unit was suggested to be located at $C_{3'}$. Based on the ^{1}H-NMR, ^{13}C-NMR, DEPT and ^{1}H–^{1}H COSY spectra, the sugar unit was found to be glucose (Tables III and IV). The coupling constant (J) value for the anomeric proton was 7.5 Hz due to the $C_{1''}$ proton in α-position, and hence the compound is a β-glucoside. Compound **3**, was refluxed with 5% aqueous HCl to yield an aglycone, which was found to be **1** by comparison of the ^{1}H-NMR spectra. The sugar obtained after hydrolysis in the aqueous layer was confirmed to be glucose by GC analysis based on a comparison of the retention time with that of a standard sample of glucose after trimethylsilylation. Furthermore, the ^{1}H-NMR signals at δ_H 6.89 and 6.98 were both broad-singlet and their assignment was due to the NOE experiment. On irradiation the anomaric proton signal of glucose at δ_H 5.86 gave the enhancement of the peaks at δ_H 6.89 and 6.45 in the NOE difference spectrum which were assigned to be the $C_{2'}$-H and $C_{4'}$-H, respectively. Thus, the structure of **3** was assigned as moracin M-3′-*O*-β-D-glucopyranoside.

The other known compounds (ursolic acid, kaempferol-3-*O*-β-glucopyranoside, quercetin-3-*O*-β-glucopyranoside, β-sitosterol, and β-sitosterol-3-*O*-β-glucopyranoside) were identified by comparing ^{1}H- and ^{13}C-NMR data with those reported in the literature as well as with the spectra of authentic samples and their derivatives. They were further confirmed by 2D NMR spectra. These known compounds have not been found previously in this plant.

Examination of the EtOAc- and *n*-BuOH-soluble fractions afforded 2-arylbenzofuran-type compounds (**1**, **2** and **3**) and flavonoid glucosides (kaempferol-3-*O*-β-glucopyranoside, quercetin-3-*O*-β-glucopyranoside). The hypoglycemic activity of these compounds has not been examined yet owing to insufficient availability for animal experiments *in vivo*, and further separation is in progress.

Experimental

All melting points were determined with a Kofler type apparatus without correction. IR spectra were taken on a Hitachi 260-10 infrared spectrophotometer in KBr disc and absorbance frequency is expressed in cm^{-1}. UV spectra were taken on a Shimadzu UV 2200 ultravoilet-visible spectrophotometer in MeOH and the λ_{max} is expressed in nanometers (nm). Optical rotation was measured on a JASCO DIP-4 automatic polarimeter at 26 °C. ^{1}H- and ^{13}C-NMR spectra were taken on JEOL GX-400 and JNM-FX 90Q Fourier-transform NMR spectrometers with TMS as an internal standard for ^{1}H-NMR and chemical shifts are expressed in δ-values. ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY and HMBC spectra were obtained with the usual pulse sequences and data processing was performed with the standard JEOL software. MS and high-resolution MS were taken on a JEOL JMX DX-300 mass spectrometer using a direct inlet system. Glycerol was used as the matrix in fast atomic bombardment MS measurements. Gas chromatography was carried out on a Shimadzu GC-6 AM gas chromatogram under the following conditions: Silicon OV-1, 3%, (2 m × 3 mm), injection temperature, 230 °C, column temperature, 180 °C, carrier gas N_2. The rat blood glucose analysis was carried out on a Reflotron kit using standard Reflotron Glucose strip (Boenhringer Mannheim Toho) based on the glucose oxidation method. Column chromatography was done with Wako gel C-200 (Wako Pure Chemical Co., Osaka, Japan) and TLC and preparative TLC were carried out on precoated Merck Kieselgel F_{254} plates (0.25 or 0.5 mm). Other chemicals [streptozotocin (Sigma), heparin (Wako, Japan), tolbutamide (Chugai, Japan), and buformine (Kodama, Japan)] were of analytical grade.

Extraction and Isolation The leaves of *M. insignis* were collected in Argentina, a voucher sample has been deposited in the museum of Toyama Medical and Pharmaceutical University, Toyama, Japan. Shade-dried powdered leaves (5 kg) were refluxed with 70% aqueous EtOH for 3 h in the first extraction and 2 h each in the 2nd and 3rd extractions. The total filtrate (81 l) was evaporated under reduced pressure to obtain a dark green viscous mass (600 g). From this extract, 185 g was suspended in distilled water (1.5 l) and extracted with EtOAc (5 × 1 l). Concentrating of the EtOAc layer (5 l) *in vacuo* yielded the EtOAc-soluble fraction (80 g). The aqueous layer was again extracted with *n*-BuOH (3 × 1 l) to give the *n*-BuOH soluble-fraction (34 g) after concentration of the *n*-BuOH layer (3 l) *in vacuo*. The aqueous layer was then lyophilized after concentration to obtain the water-soluble fraction (69 g).

The EtOAc-soluble fraction (77 g) was applied to a Silica gel G (60—230 mesh; 1.5 kg) column and eluted with CH_2Cl_2 and a mixture of CH_2Cl_2–MeOH increasing the polarity by increasing the concentrations of MeOH, and fractions of about 500 ml each fraction were collected. Fractions 16—24 yielded β-sitosterol (1.5 g) after recrystallization from benzene–hexane mixture, and ursolic acid (640 mg) was obtained from fr. 50 after rechromatography on silica gel with hexane chloroform mixture. The rechromatography of fr. 68 (obtained by eluting 20% MeOH in CH_2Cl_2) of the EtOAc-soluble fraction gave moracin M (14 mg) alone and as a mixture with **2**. The mixture was subjected to preparative silica gel TLC with the two solvent systems, a) MeOH : $CHCl_3$ (1 : 9) and b) acetone : hexane (2 : 3), to give slightly impure **2**. This was further purified by preparative silica gel TLC with the solvent system acetone : benzene (1 : 9) run seven times to give **2** (8 mg). Fractions 70—75, on silica gel column chromatography with MeOH–$CHCl_3$ mixture followed by attempted crystallization (abortive) from MeOH–$CHCl_3$ mixture, afforded β-sitosterol-3-*O*-β-glucopyranoside (1.2 g). Kaempferol-3-*O*-β-glucopyranoside (10 mg) and quercetin-3-*O*-β-glucopyranoside (11 mg) were obtained by cellulose column chromatography followed by preparative TLC. The *n*-BuOH-soluble fraction (10 g) was applied to a Sephadex LH-20 column (2 cm × 94 cm) and eluted with water and a mixture of water–MeOH, increasing concentrations of MeOH. Repeated Sephadex LH-20 column chromatography with MeOH : H_2O yielded kaempferol-3-*O*-β-glucopyranoside (40 mg), quercetin-3-*O*-β-glucopyranoside (82 mg) and a moracin M-3′-*O*-β-D-glucopyranoside (**3**)-containing fraction, which was purified by preparative silica gel TLC with the solvent system MeOH : $CHCl_3$ (3 : 7) followed by crystallization give **3** (10 mg).

β-Sitosterol: White needles, $C_{29}H_{50}O$. ^{13}C-NMR (22.5 MHz, $CDCl_3$): δ_C 140.86, 121.74, 71.84, 56.89, 56.19, 50.28, 46.00, 42.43, 39.93, 37.39, 36.63, 36.25, 34.08, 32.03, 31.76, 29.31, 28.34, 26.28, 24.44, 23.20, 21.19, 19.95, 19.51, 19.18, 18.92, 11.98. EI-MS m/z: 414 (M^+), 396 ($M^+ - H_2O$), 329, 355, 213, 145, 81.

Ursolic Acid: White crystalline solid, $C_{30}H_{46}O_3$. ^{1}H-NMR (400 MHz, pyridine-d_5): δ_H 5.50 (1H, t, $J=4.0$ Hz), 3.46 (1H, m), 2.63 (1H, br d, $J=11.5$ Hz), 2.33 (1H, ddd, $J=14.0$, 13.0, 5.0 Hz), 2.13 (1H, ddd, $J=14.0$, 13.0, 5.0 Hz), 2.02 (1H, br d, $J=3.5$ Hz), 1.94—1.97 (4H, m), 1.82 (2H, m), 1.65 (1H, br d, $J=9.0$ Hz), 1.53—1.62 (4H, m), 1.45—1.50 (2H, m), 1.35—1.40 (4H, m), 1.29—1.30 (2H, m), 1.25 (3H, s), 1.24 (3H, s), 1.06

(3H, s), 1.03 (3H, s), 1.01 (3H, d, $J=6.0$ Hz), 0.97 (3H, d, $J=6.0$ Hz), 0.91 (3H, s). ^{13}C-NMR (100 MHz, pyridine-d_5): δ_C 178.2 (s), 139.3 (s), 125.6 (d), 78.2 (d), 55.9 (d), 53.6 (d), 48.1 (d), 42.5 (s), 40.0 (s), 39.4 (d), 39.2 (t), 37.4 (t), 37.3 (t), 33.6 (t), 31.1 (t), 28.8 (q), 28.1 (t), 24.9 (t), 23.9 (q), 23.7 (t), 21.4 (q), 17.5 (t), 16.5 (q), 15.7 (q). EI-MS m/z: 456 (M^+), 438 (M^+-H_2O), 410, 355, 248, 207, 203, 127.

β-Sitosterol-3-O-β-glucopyranoside: Amorphous powder. ^{1}H-NMR (400 MHz, pyridine-d_5): δ_H 6.38 (1H, m, C_6-H), 5.03 (1H, d, $J=8.0$ Hz, $C_{1'}$-H), 4.55 (1H, dd, $J=11.5$, 2.5 Hz, $C_{6'}$-H), 4.38 (1H, dd, $J=11.5$, 5.5 Hz, $C_{6'}$-H), 4.31 (1H, t, $J=8.0$ Hz, $C_{3'}$-H), 4.24 (1H, t, $J=8.0$ Hz, $C_{4'}$-H), 4.05 (1H, t, $J=8.0$ Hz, $C_{2'}$-H), 3.99 (1H, m, C_3-H), 3.98 (1H, m, $C_{5'}$-H), 1.00 (3H, d, $J=6.5$ Hz, C_{21}-H), 0.94 (3H, s, C_{19}-H), 0.90 (3H, t, $J=7.0$ Hz, C_{29}-H), 0.86, 0.89 (3H each, d, $J=7.0$ Hz, C_{26}-H or C_{27}-H), 0.67 (3H, s, C_{18}-H).

Acetylation of β-Sitosterol-3-O-β-glucopyranoside: β-Sitosterol-3-O-β-glucopyranoside (20 mg) was treated with a mixture of the Ac_2O and pyridine at room temperature for 15 h then the mixture was extracted with $CHCl_3$ and purified by preparative TLC to give the acetate of β-sitosterol-3-O-β-glucopyranoside (24 mg). ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H 5.36 (1H, m, C_6-H), 5.20 (1H, t, $J=9.5$ Hz, $C_{3'}$-H), 5.08 (1H, t, $J=9.8$ Hz, $C_{4'}$-H), 4.96 (1H, dd, $J=9.5$, 7.9 Hz, $C_{2'}$-H), 4.59 (1H, d, $J=7.9$ Hz, $C_{1'}$-H), 4.26 (1H, dd, $J=12.2$, 4.8 Hz, $C_{6'}$-H), 4.11 (1H, dd, $J=12.2$, 2.4 Hz, $C_{6'}$-H), 3.68 (1H, ddd, $J=9.8$, 4.8, 2.4 Hz, $C_{5'}$-H), 3.49 (1H, m, C_3-H), 2.00—2.08 (3H×4, each s, $COCH_3\times4$), 0.99 (3H, s, C_{19}-H), 0.92 (3H, d, $J=6.4$ Hz, C_{21}-H), 0.85 (3H, t, $J=7.6$ Hz, C_{29}-H), 0.84 (3H, d, $J=7.3$ Hz, C_{27}-H or C_{26}-H), 0.81 (3H, d, $J=7.0$ Hz, C_{26}-H or C_{27}-H), 0.68 (3H, s, C_{18}-H).

Moracin M (**1**): Light pink crystalline solid, mp 275 °C, $C_{14}H_{10}O_4$. HR-MS: Found 242.0561, Calcd for $C_{14}H_{10}O_4$ was 242.0578. UV λ_{max} nm (ε): 214 (9837), 304 (10125), 318 (11365). IR ν_{max} cm^{-1}: 3525, 3300 (br), 1615, 1580, 1440, 1365, 1295, 1145, 1126, 1008, 970, 822. ^{1}H- and ^{13}C-NMR see in Tables III and IV. EI-MS m/z: 242 (M^+), 213, 121, 69.

Mulberrofuran U (**2**): Light yellow amorphous powder, $C_{39}H_{36}O_9$, mp 286 °C (dec.). $[\alpha]_D$ +128° (MeOH, $c=0.13$). UV λ_{max} nm (ε): 207 (40355), 293 (20561), 318 (28576), 332.5 (23285). IR ν_{max} cm^{-1}: 3400, 1625, 1505, 1440, 1380, 1320, 1275, 1235, 1110, 1030, 980, 830. ^{1}H- and ^{13}C-NMR see Tables III and IV. Positive ion FAB-MS m/z: 649 $(M+H)^+$ and 671 $(M+Na)^+$. Negative ion FAB-MS m/z: 647 $(M-H)^-$. HR-MS: Found 671.2249, Calcd for $C_{39}H_{36}O_9Na$ 671.2257.

Kaempferol-3-O-β-glucopyranoside: Yellow amorphous powder. ^{1}H-NMR (400 MHz, DMSO-d_6): δ_H 8.04 (2H, d, $J=9.0$ Hz, $C_{2'}$-H, $C_{6'}$-H), 6.88 (2H, d, $J=9.0$ Hz, $C_{3'}$-H, $C_{5'}$-H), 6.42 (1H, d, $J=2.0$ Hz, C_8-H), 6.20 (1H, d, $J=2.0$ Hz, C_6-H), 5.45 (1H, d, $J=7.0$ Hz, $C_{1''}$-H), 5.26 (1H, br s, $C_{2''}$-OH), 5.02 (1H, br s, $C_{3''}$-OH), 4.86 (1H, br s, $C_{4''}$-OH), 4.22 (1H, br s, $C_{6''}$-OH), 3.56 (1H, br d, $J=12.0$ Hz, C_6-H_a), 3.34 (1H, br d, $J=12.0$ Hz, $C_{6''}$-H_b), 3.25 (2H, m, $C_{2''}$-H, $C_{3''}$-H), 3.06 (2H, br, $C_{4''}$-H, $C_{5''}$-H). ^{13}C-NMR (100 MHz, DMSO-d_6): δ_C 177.4 (s, C_4), 164.0 (s, C_7), 161.2 (s, C_5), 159.9 (s, $C_{4'}$), 156.4 (s, C_9), 156.1 (s, C_2), 133.1 (s, C_3), 130.8 (d, $C_{2'}$, $C_{6'}$), 120.9 (s, $C_{1'}$), 115.1 (d, $C_{3'}$, $C_{5'}$), 103.9 (s, C_{10}), 100.9 (d, $C_{2''}$), 98.7 (d, C_6), 93.7 (d, C_8), 77.4 (d, $C_{3''}$), 76.4 (d, $C_{5''}$), 74.2 (d, $C_{2''}$), 69.8 (d, $C_{4''}$), 60.8 (t, $C_{6''}$).

Moracin M-3′-O-β-D-glucopyranoside (**3**): Pale yellow crystalline solid, $C_{20}H_{20}O_9$, mp 284 °C. It gave a single spot on the silica gel-coated TLC plate with the following solvent systems $CHCl_3$: MeOH : H_2O (16 : 8 : 1) ($Rf=0.4$), MeOH : $CHCl_3$ (3 : 7) ($Rf=0.5$) and EtOAc : AcOH : H_2O (100 : 15 : 12) ($Rf=0.7$) with UV fluorescence detections as well as $Ce(SO_4)_2/H_2SO_4$ spray reagent. It was levorotatory, $[\alpha]_D$ −43.1° (MeOH, $c=0.87$). UV λ_{max} nm (ε): 216.5 (10971), 315 (11862), 327 (10355). IR ν_{max} cm^{-1}: 3350, 2900, 1615, 1600, 1570, 1480, 1425, 1350, 1280, 1265, 1195, 1160, 1030, 960, 835, 810. ^{1}H- and ^{13}C-NMR see Tables III and IV. Positive ion FAB-MS m/z: 405 $(M+H)^+$.

Quercetin-3-O-β-glucopyranoside: Yellow amorphous powder, $C_{21}H_{20}O_{12}$. ^{1}H-NMR (400 MHz, DMSO-d_6): δ_H 7.61 (1H, br s, $C_{2'}$-H), 7.60 (1H, dd, $J=9.0$, 2.5 Hz, $C_{6'}$-H), 6.88 (1H, d, $J=9.0$ Hz, $C_{5'}$-H), 6.44 (1H, d, $J=2.5$ Hz, C_6-H), 6.23 (1H, d, $J=2.5$ Hz, C_8-H), 5.50 (1H, d, $J=7.5$ Hz, $C_{1''}$-H), 5.35 (1H, br s, $C_{2''}$-OH), 5.13 (1H, br s, $C_{3''}$-OH), 5.02 (1H, br s, $C_{4''}$-OH), 4.33 (1H, t, $J=5.5$ Hz, $C_{6''}$-OH), 3.62 (1H, br d, $J=12.0$ Hz, $C_{6''}$-H_a), 3.35 (1H, br d, $J=12.0$ Hz, $C_{6''}$-H_b), 3.26 (2H, br d, $C_{2''}$-H, $C_{3''}$-H), 3.13 (2H, br, $C_{4''}$-H, $C_{5''}$-H). ^{13}C-NMR (100 MHz, DMSO-d_6): δ_C 177.6 (s, C_4), 164.2 (s, C_7), 161.4 (s, C_5), 156.4, 156.4 (s, C_2, C_9), 148.6 (s, $C_{4'}$), 144.0 (s, $C_{3'}$), 133.5 (s, C_3), 122.1 (d, $C_{6'}$), 121.8 (s, $C_{1'}$), 116.4 (d, $C_{5'}$), 115.4 (d, $C_{2'}$), 104.2 (s, C_{10}), 101.0 (d, $C_{1''}$), 98.8 (d, C_6), 93.7 (d, C_8), 77.7 (d, $C_{3''}$), 76.6 (d, $C_{5''}$), 74.2 (d, $C_{2''}$), 70.0 (d, $C_{4''}$), 61.1 (t, $C_{6''}$).

Acid Hydrolysis of Moracin M-3′-O-β-D-Glucopyranoside (3) Moracin M-3′-O-β-D-glucopyranoside (7 mg) was refluxed with 5% aqueous HCl (1 ml) for 2 h in a boiling water bath. Distilled water (5 ml) was added to the reaction mixture, then it was extracted with EtOAc (5 ml × 5). The EtOAc-soluble fraction was dried with anhydrous Na_2SO_4 and purified by silica gel preparative TLC with the solvent system MeOH : $CHCl_3$ (1 : 9) to give the aglycone (3 mg), which was identified as moracin M (**1**) by comparison of the ^{1}H-NMR spectrum with that of an authentic sample. The sugar was identified as glucose by GC analysis after trimethylsilylation, in comparison with an authentic sample.

Animals and Treatment Male Sprague-Dawley rats, 5 weeks old, weighing 120—140 g were purchased from the Shizuoka Laboratory Animal Center (Japan) and maintained under a 12 h light/dark cycle in a temperature- and humidity-controlled room. The animals were fed with CE 2 (Clea, Japan) and given water *at libitum*. Diabetes was induced in the 16 h fasted rats by a single intravenous injection of 50 mg/kg streptozotocin (STZ) in a citrate buffer (pH 4.5) according to the protocol of Like and Rossini (1976).[18] The blood glucose level was checked on the third and fourth days after injecting the STZ. The animals having high blood glucose (more than 300 mg/dl) were divided into groups. Drugs or physiological saline were administered to each group orally or intraperitoneally. Blood samples were collected 6 h after administering the last dose (i.p.) in the case of continuous treatment (Fig. 1). Blood was sampled with a syringe through the jugular vein and immediately transferred into a tube which had been rinsed with heparin. The glucose level in the blood sample were analyzed within an hour by using Reflotron kits.

Statistical Analysis All values are expressed as mean±S.E. (n experiments). Student's t-test for unpaired observations was used for statistical evaluation of the differences; p values of 0.05 or less were considered as significant.

Acknowledgement We are grateful to Prof. Taro Nomura, Faculty of Pharmaceutical Science, Toho University, Japan for valuable discussions. We also thank Dr. Katsuhide Matoba and Mr. Masakazu Nagasawa, Research Institute Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan for high resolution MS, and Mr. Y. Kawata, Toyama Medical and Pharmaceutical University, Toyama, Japan for MS measurement.

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