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JAVABERINE A, NEW TNF-α AND NITRIC OXIDE PRODUCTION INHIBITOR, FROM THE ROOTS OF *TALINUM PANICULATUM*

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Abstract — The methanolic (MeOH) extract from the roots of *Talinum paniculatum* was found to exhibit inhibitory activities on the lipopolysaccharide (LPS)-induced tumor necrosis factor (TNF)-α and nitric oxide (NO) production from mouse peritoneal macrophages, and on the TNF-α production from rat epididymal adipose tissue. From the MeOH extract, two new 8-benzyltetrahydroprotoberberine–type alkaloids, named javaberines A and B, were isolated and their structures were elucidated on the basis of chemical and physicochemical evidence. The principal alkaloid, javaberine A, inhibited LPS-induced TNF-α and NO production from mouse peritoneal macrophages and TNF-α production from rat epididymal adipose tissue.

The Portulacaceae plant *Talinum paniculatum* GAERTNER, which is commonly called "Ginseng Java" or "Som Java" in Indonesia, is widely distributed in China, South Eastern Asia, and in South American countries. The roots of this plant are used as a tonic for men, a cure for pneumonitis, and for improving menostasia, while the fresh leaves are consumed as a vegetable. On the other hand, the roots of *T. paniculatum* are used for the treatment of neuralgia and arthritis in Brazil. Chemical constituents from the roots including higher alcohols (1-hexacosanol, 1-octacosanol, and 1-triacontanol), their acetates, sterols (campesterol, stigmasterol, and β-sitosterol), and β-sitosterol-glucoside were reported previously.[1] However, the pharmacologically active constituents of this natural medicine are left uncharacterized.

As a part of our studies on tumor necrosis factor (TNF)-α and nitric oxide (NO) production inhibitors from natural medicines[2] and medicinal foodstuffs,[3] we found that the methanolic (MeOH) extract from the roots of *T. paniculatum* inhibited lipopolysaccharide (LPS)-induced TNF-α and NO production from

mouse peritoneal macrophages, and TNF-α production from rat epididymal adipose tissue. In this communication, we describe the isolation and structure elucidation of two novel 8-benzyltetrahydroprotoberberines, javaberines A (**1**) and B (**2**), from the roots of Indonesian *T. paniculatum*.as well as the inhibitory activities of **1** on TNF-α and NO production.

Although, inflammatory cytokines (*e.g.* TNF-α) and NO produced from activated phagocytes act on host defense, excessive production causes damage to host cells. Sepsis is one of the serious pathosis induced by acute facilitation of TNF-α and NO production from macrophages that can cause shock and death. On the other hand, serum TNF-α levels in patients with immunological diseases such as rheumatoid arthritis[4] and Crohn's disease[5] are reported to be high. NO is also an important mediator of various types of inflammation.[6] TNF-α and NO, produced from activated phagocytes, are considered to be important mediators in inflammatory diseases.

Recently, TNF-α produced from adipose tissue was reported to be involved in insulin resistance in adipocytes.[7] An increase in fat mass enhances the amount of TNF-α secreted from adipose tissue. Actually, the plasma TNF-α levels are reported to correlate with the amount of visceral fat in type 2 diabetic patients.[8] This evidence suggests that the production of TNF-α from fat tissue is a key factor of worsening diabetes with obesity.

In this study of the anti-inflammatory activity of *T. paniculatum*, we investigated the effect of an MeOH extract from the roots on LPS-induced TNF-α and NO production from mouse peritoneal macrophages. The cells (5×10^5 cells/100 μL) suspended in RPMI-1640 containing 5% fetal calf serum (FCS) were seeded in a 96-well plate. After 1 h culture, medium was removed with the non-adherent cells and the wells were washed with phosphate-buffered saline (PBS). New medium (200 μL) containing sample and LPS (10 μg/mL) was added to each well, and the plate was continuously cultured for 4 h (TNF-α) or 20 h (NO). After the culture, medium was collected and its TNF-α and NO_2^- (oxidised product of NO) contents were measured

Table 1. Effects of the MeOH Extract from the Roots of *T. paniculatum* on LPS-induced TNF-α and NO Production from Mouse Peritoneal Macrophages

		TNF-α production		NO production	
	Conc.	Amount	Inhibition	Amount	Inhibition
	(μg/mL)	(pg/mL)	(%)	(NO_2^-, μM)	(%)
Non-stimulated	—	0.1±1.3**	—	0.8±2.9**	—
Control (LPS-stimulated)	—	151.1±4.6	—	35.1±3.0	—
MeOH extract	1	NT	—	28.6±3.9	19
	3	95.3±11.4**	37	26.2±4.0**	26
	10	79.6±8.8**	47	23.6±1.9**	34
	30	78.3±3.6**	48	20.6±2.3**	42
	100	60.1±7.6**	60	13.4±3.5**	63

Each value represents the mean with S.E.M. of 4 experiments. Significant difference from the control: $^{**}p<0.01$. NT: not tested.

by enzyme-linked immunosorbent assay (ELISA, Amersham Pharmacia Biotec) and Griess method,[9] respectively. As a result, the MeOH extract (yield: 5.6%) suppressed both TNF-α and NO production in a concentration-dependent manner from 3 to 100 μg/mL (Table 1).

We also examined the effect of the MeOH extract on LPS-induced TNF-α production from rat epididymal adipose tissue.[10] Rat epididymal fat (10 g) was cut into small pieces (1–2 mm square) in 40 mL of Medium 199 containing 10% FCS. After incubation (37 °C, 10 min), the sample was centrifuged (180 ×*g*, rt, 10 min) and the infranatant was removed. Fat debris was suspended in new medium (40 mL) and put into a 24-well plate (500 μL/well). Sample solution (100 μL) and 25 μg/mL LPS (400 μL) were added, and the plate was incubated (37 °C, 20 h). The plate was centrifuged (180 ×*g*, 10 min) and the amount of TNF-α in the infranatant was determined by the method described above. As shown in Figure 1, the MeOH extract from *T. paniculatum* significantly suppressed TNF-α production from fat tissue at concentrations of 10 and 30 μg/mL.

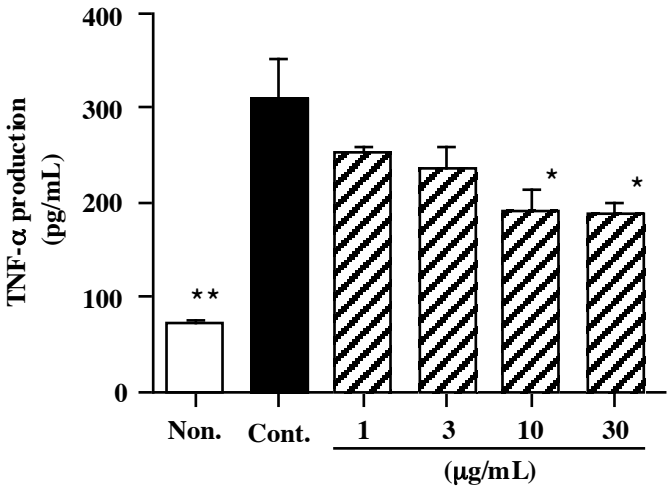
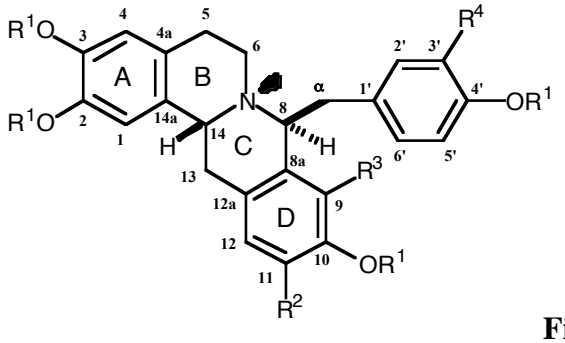


Figure 1. Inhibitory Effects of the MeOH Extract from the Roots of *T. paniculatum* on LPS-induced TNF-α Production from Rat Epididymal Adipose Tissue

Each column represents the mean with S.E.M. of 4 experiments. Significantly difference from the control: *p<0.05, **p<0.01, respectively. Non.: non LPS-stimulation, Cont.: LPS-stimulation.

To characterize the active constituents in the roots of *T. paniculatum*, the MeOH extract was subjected to the following separation procedure: The MeOH extract w as successively extracted with AcOEt, *n*-BuOH, and H_2O. The *n*-BuOH soluble-portion (1.2%) was separated by normal-phase silica gel column chromatography [$CHCl_3$–MeOH–H_2O (10 : 3 : 0.5 → 7 : 3 : 0.5 → 6 : 4 : 1, v/v) → MeOH], reversed-phase silica gel column chromatography [MeOH–H_2O (0 : 100 → 30 : 70 → 70 : 30 → 100 : 0, v/v)], and finally Toyopearl HW-40F column chromatography (H_2O → MeOH) to give javaberine A (**1**, 0.026%). Furthermore, the minor alkaloid fraction was subjected to acetylation reaction with Ac_2O–pyridine mixture. The acetylated mixture was purified by preparative HPLC [ODS, MeCN–H_2O (60 : 40, v/v), UV (280 nm)] to give javaberines A hexa-*O*-acetate (**1a**, 0.0019%) and B hexa-*O*-acetate (**2a**, 0.0011%) and 8-(*p*-hydroxybenzyl)-2,3,10,11-tetrahydroxyprotoberberine penta-*O*-acetate (**3**,[11] 0.00040%) (Figure 2).

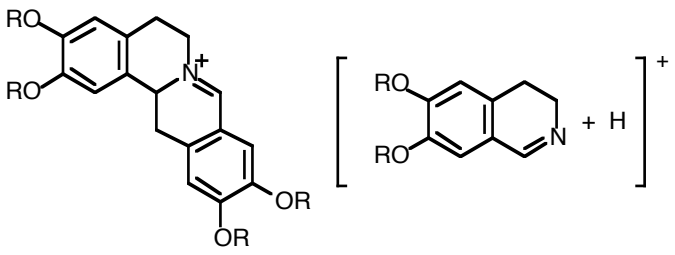
Javaberine A (**1**),[12] a white powder, $[\alpha]_D^{25}$ +6.6° (*c*=0.5, MeOH), $C_{24}H_{23}NO_6$, showed absorption bands at 3389, 1620, 1528, and 1385 cm^{-1} assignable to hydroxyl and aromatic functions in the IR spectrum,



	R^1	R^2	R^3	R^4
javaberine A (**1**) :	H	OH	H	OH
javaberine A acetate (**1a**) :	Ac	OAc	H	OAc
javaberine B (**2**) :	H	H	OH	OH
javaberine B acetate (**2a**) :	Ac	H	OAc	OAc
3 :	Ac	OAc	H	H

Figure 2

while its UV spectrum indicated the presence of phenolic chromophores from the absorption maximum at 287 nm (log ε 3.9). Acetylation of **1** with Ac_2O in pyridine yielded the hexaacetyl derivative (**1a**).[13] The ^{1}H- and ^{13}C-NMR (**1**, CD_3OD; **1a**, $CDCl_3$) spectra of **1** and **1a**, which were assigned with the aid of various NMR analytical methods,[14] showed signals due to three methylenes [**1**: δ 2.92—3.00 (2H, m, 5-H_2), 2.86 (dd, *J* = 11.4, 17.8 Hz), 3.25 (m) (13-H_2), 3.39, 3.48 (both m, 6-H_2); **1a**: δ 2.75, 2.95 (both m, 5-H_2), 2.75, 3.06 (both m, 6-H_2), 2.88 (dd, *J* = 5.3, 17.2 Hz), 2.95 (dd, *J* = 11.0, 17.2 Hz) (13-H_2)], two methines [**1**: δ 4.46 (br d, *J* = 7.3 Hz, 8-H), 4.81 (m, 14-H); **1a**: δ 3.95 (dd, *J* = 5.5, 7.6 Hz, 8-H), 4.40 (dd, *J* = 5.3, 11.0 Hz, 14-H)], a 3,4-dihydroxyl or diacetoxybenzyl group [**1**: δ 2.97, 3.31 (each m, α-H_2), 6.39 (br d, *J* = 7.8 Hz, 6'-H), 6.50 (br s, 2'-H), 6.70 (d, *J* = 7.8 Hz, 5'-H); **1a**: δ 2.82 (dd, *J* = 5.5, 13.7 Hz), 3.18 (dd, *J* = 7.6, 13.7 Hz) (α-H_2), 7.02 (br s, 2'-H), 7.06 (d, *J* = 8.2 Hz, 5'-H), 7.08 (br d, *J* = 8.2 Hz, 6'-H)], and two tetrasubstituted benzene rings [**1**: δ 5.92 (s, 9-H), 6.58 (s, 12-H), 6.63 (s, 4-H), 6.65 (s, 1-H); **1a**: δ 6.64 (s, 9-H), 6.90 (s, 12-H), 6.95 (s, 4-H), 6.97 (s, 1-H)]. The chemical shifts of the 6-C (δc 47.1, 46.2), 8-C (δc 68.3, 66.2), and 14-C (δc 53.7, 50.2) in the ^{13}C-NMR spectra of 1 and 1a coincided with the fact that these carbons were adjacent to nitrogen atom. The positive-ion FAB-MS of **1** and **1a** showed intense fragment ion peaks (**i**, **ii**) at *m/z* 298 and 466, respectively, which were generated by debenzylation at the 8-position,[15] whereas the fragment ion peaks (**iii**, **iv**) were also observed at *m/z* 164 and 248, which were produced *via* characteristic retro-Diels-Alder fragmentationof the C-ring in tetrahydroprotoberberine type alkaloids (Figure 3).[16] The 8-benzyltetrahydroprotoberberine skeleton of **1** was characterized by the heteronuclear multiple bond connectivity (HMBC) experiments and ^{1}H-^{1}H COSY experiments on **1** and **1a** as shown in Figure 4. Furthermore, in a differential NOE experiment on **1a**, NOE correlations were observed between the 14 proton and the methylene protons (α-H_2) of the 3,4-dihydroxybenzyl group, thus the stereostructure of **1** was determined.



i : R = H (*m/z*298)
ii : R = Ac (*m/z* 466)

iii : R = H (*m/z* 164)
iv : R = Ac (*m/z* 248)

Figure 3

The conformation of tetrahydroprotoberberines was analyzed by ^{1}H- and ^{13}C-NMR ($CDCl_3$) examination. The B/C-*trans* conformations are associated with high-field 14-proton signals at 3.6±0.2 ppm and low-field 14-carbon signals at 58.4±0.3 ppm, while the 14-protons are shifted to low-field at 4.3±0.2 ppm and the 14-carbons to high-field at about 49—52 ppm in the B/C-*cis* conformation.[17] Furthermore, the 6-carbon signal of the B/C-*trans* conformation was reported to appear at δc 51.0—51.6.[18] The proton signal of the 14-position in the ^{1}H-NMR ($CDCl_3$) spectrum of **1a** was observed at δ 4.40, while the 14- and 6-carbons of **1a** in the ^{13}C-NMR ($CDCl_3$) spectrum appeared at δc 50.2 and 46.2, respectively. This evidence, and the absence of Bohlmann bands in the IR spectra of **1** and **1a**, indicated the B/C-*cis* conformation of **1**. Consequently, the stereostructure of javaberine A (**1**) was elucidated as shown.

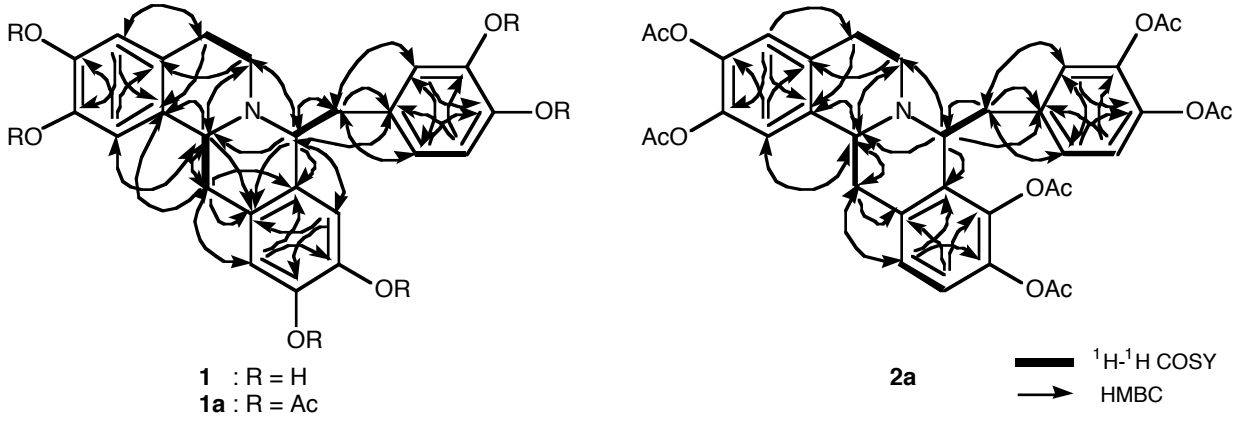


Figure 4. HMBC and ^{1}H-^{1}H COSY Correlations of Javaberines (**1**, **1a**, and **2a**)

Javaberine B (**2**) was obtained as the hexaacetate (**2a**),[19] which had the same molecular formula $C_{36}H_{35}NO_{12}$ as that of **1a.** The IR, UV, and MS spectra of **2a** were very similar to those of **1a**. The proton and carbon signals in the ^{1}H- and ^{13}C-NMR spectra of **2a** were superimposable on those of **1a**, except for the signals due to the D-ring. That is, the ^{1}H- and ^{13}C-NMR ($CDCl_3$) spectra of **2a** indicated the presence of a 3,4-diacetoxybenzyl group [δ 2.74 (dd, *J* = 3.1, 14.3 Hz), 3.09 (dd, *J* = 9.2, 14.3 Hz) (α-H_2), 7.07 (d, *J* = 8.2 Hz, 5'-H), 7.11 (d, *J* = 1.8 Hz, 2'-H), 7.15 (d, *J* = 1.8, 8.2 Hz, 6'-H)], a protoberberine moiety [δ 2.65 (m, 5-H, 6-H), 2.88 (dd, *J* = 5.5, 17.4 Hz), 2.97 (dd, *J* = 11.3, 17.4 Hz) (13-H_2), 2.97 (m, 5-H, 6-H), 3.94 (dd, *J* = 3.1, 9.2 Hz, 8-H), 4.49 (dd, *J* = 5.5, 11.3 Hz, 14-H), 6.95 (s, 4-H), 6.99 (s, 1-H), 7.01 (d, *J* = 8.2 Hz, 12-H), 7.06 (d, *J* = 8.2 Hz, 11-H)], and six acetyl groups [δ 2.25, 2.26, 2.27, 2.28, 2.29, and 2.30 (all s)]. The planar structure of **2a** was elucidated by HMBC experiment as shown in Figure 4. The stereostructure of **2a** was characterized by differential NOE experiment, which showed a correlation between the 14-proton and the α-methylene protons of the 3,4-diacetoxybenzyl group. Examination of the chemical shift values of the 14-proton and carbon as well as the 6-carbon led us to confirm the B/C-*cis* conformation of **2a**. On the basis of this evidence, the stereostructure of javaberine B acetate (**2a**) was determined as shown.

Finally, we examined the inhibitory activity of javaberine A (**1**) on LPS-induced TNF-α and NO production from mouse peritoneal macrophages. It was found that **1** potently inhibited TNF-α production from 1 to 100 μM and NO production from 3 to 100 μM (Table 2). Hydrocortisone is reported to inhibit LPS-induced NO production from J774 cells with IC_{50} value of 5 μM.[20] The IC_{50} value of **1** against NO production is approximately 20 μM on peritoneal macrophage. The potential of **1** appeared to be close to that of the steroidal medicine.

Table 2. Effects of Javaberine A (**1**) on LPS-induced TNF-α and NO Production from Mouse Peritoneal Macrophages

		TNF-α production		NO production	
	Conc.	Amount	Inhibition	Amount	Inhibition
	(μM)	(pg/mL)	(%)	(NO_2^-, μM)	(%)
Non-stimulated	—	0.1±1.3**	—	0.8±1.9**	—
Control (LPS-stimulated)	—	151.1±4.6	—	40.3±3.1	—
Javaberine A (**1**)	1	NT	—	28.4±2.9**	30
	3	88.7±6.9**	41	24.3±3.3**	41
	10	65.3±3.4**	57	21.6±1.1**	47
	30	48.6±2.6**	68	17.2±3.6**	58
	100	40.9±2.2**	73	10.4±2.6**	76

Each value represents the mean with S.E.M. of 4 experiments. Significantly different from the control: $^{**}p<0.01$. NT: not tested.

Moreover, we investigated the effect of javaberine A (**1**) on LPS-stimulated TNF-α production from rat epididymal adipose tissue. As shown in Figure 5, javaberine A (**1**) significantly inhibited TNF-α production at 10 μM. A thiazolidinedione, BRL49653, is reported to show almost 90% inhibition at 0.1 μM against TNF-α production from human adipose tissue.[10] Although **1** appears to be less effective than synthetic thiazolidinedione derivatives, this is the first report that this type alkaloids exhibit inhibitory activity against TNF-α production from adipose tissue.

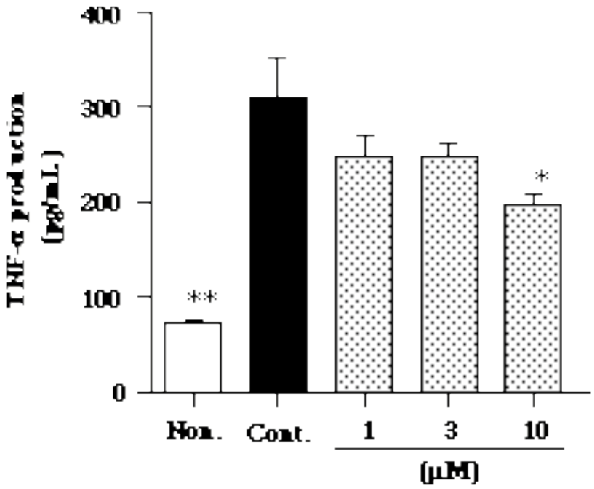


Figure 5. Inhibitory Effects of Javaberine A (**1**) on LPS-induced TNF-α Production from Rat Epididymal Adipose Tissue

Each column represents the mean with S.E.M. of 4 experiments. Significantly difference from the control: $^{*}p<0.05$, $^{**}p<0.01$, respectively. Non.: non LPS-stimulation, Cont.: LPS-stimulation.

In conclusion, we isolated new alkaloids including javaberine A (**1**) that inhibited TNF-α and NO production from macrophages and TNF-α production from adipose tissue. Javaberine A (**1**) might be a useful compound for the prevention of diabetes with obesity through inhibition of TNF-α production from fat tissue.

REFERENCES AND NOTES

1 M. Komatsu, I. Yokoe, Y. Shirataki, and T. Tomimori, *Yakugaku Zasshi*, 1982, **102**, 499.

2 H. Matsuda, K. Ninomiya, T. Morikawa, and M. Yoshikawa, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 339; H. Matsuda, T. Murakami, T. Kageura, K. Ninomiya, I. Toguchida, N. Nishida, and M. Yoshikawa, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 2191; H. Matsuda, T. Kageura, I. Toguchida, T. Murakami, A. Kishi, and M. Yoshikawa, *Bioorg. Med. Chem. Lett.*, 1999, **9**, 3081; M. Yoshikawa, T. Morikawa, I. Toguchida, S. Harima, and H. Matsuda, *Chem. Pharm. Bull.*, 2000, **48**, 651; H. Matsuda, T. Kageura, T. Morikawa, I. Toguchida, S. Harima, and M. Yoshikawa, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 323; H. Matsuda, T. Kageura, M. Oda, T. Morikawa, Y. Sakamoto, and M. Yoshikawa, *Chem. Pharm. Bull.*, 2001, **49**, 716; T. Kageura, H. Matsuda, T. Morikawa, I. Toguchida, S. Harima, M. Oda, and M. Yoshikawa, *Bioorg. Med. Chem.*, 2001, **9**, 1887; O. Muraoka, M. Fujimoto, G. Tanabe, M. Kubo, T. Minematsu, H. Matsuda, T. Morikawa, I. Toguchida, and M. Yoshikawa, *Bioorg. Med. Chem. Lett.*, 2001, **11**, in press; H. Matsuda, T. Morikawa, Y. Sakamoto, I. Toguchida, and M. Yoshikawa, *Heterocycles*, in press.

3 M. Yoshikawa, T. Murakami, H. Shimada, S. Yoshizumi, M. Saka, J. Yamahara, and H. Matsuda, *Chem. Pharm. Bull.*, 1998, **46**, 1008; H. Matsuda, T. Kageura, I. Toguchida, H. Ueda, T. Morikawa, and M. Yoshikawa, *Life Sci.*, 2000, **66**, 2151.

4 S. F. Ausar, D. M. Beltramo, L. F Castagna, S. Quintana, E. Silvera, G. Kalayan, M. Revigliono, C. A. Landa, and I. D. Bianco, *Rheumatol. Int.*, 2001, **20**, 138.

5 W. J. Sandborn, B. G. Feagan, S. B. Hanauer, D. H. Present, L. R. Sutherland, M. A. Kamm, D. C. Wolf, J. P. Baker, C. Hawkey, A. Archambault, C. N. Bernstein, C. Novak, P. K. Heath, and S. R. Targan, *Gastroenterology*, 2001, **120**, 1330.

6 H. Maeda and T. Akaike, *Biochemistry (Mosc)*, 1998, **63**, 854.

7 C. Qi and P. H. Pekala, *Proc. Soc. Exp. Biol. Med.*, 2000, **223**, 128.

8 E. Bertin, P. Nguyen, M. Guenounou, V. Durlach, G. Potron, and M. Leutenegger, *Diabetes Metab.*, 2000, **26**, 178.

9 A. H. Ding, C. F. Nathan, and D. J. Stuehr, *J. Immunol.*, 1988, **141**, 2407.

10 C. P. Sewter, J. E. Digby, F. Blows, J. Prins, and S. O' Rhilly, *J. Endocrinol.*, 1999, **163**, 33.

11 Q. Xu and M. Lin, *J. Nat. Prod.*, 1999, **62**, 1025.

12 **1**: High-resolution positive-ion FAB-MS: Calcd for $C_{24}H_{24}NO_6$ $(M+H)^+$: 422.1604. Found: 422.1609. ^{13}C-NMR (CD_3OD, 125 MHz) δc: 26.0 (5-C), 33.0 (13-C), 42.4 (α-C), 47.1 (6-C), 53.7 (14-C), 68.3 (8-C), 114.1 (1-C), 116.0 (9-C、12-C), 116.4 (4-C), 116.6 (5'-C), 118.1 (2'-C), 120.7 (8a-C), 121.5, 121.7 (4a-, 12a-C), 122.6 (6'-C), 126.0 (14a-C), 128.0 (1'-C), 145.0 (10-C), 145.7, 145.8 (4'-, 2-C), 146.4 (3'-C), 147.1 (3-, 11-C).

13 **1a**: A white powder, $[\alpha]_D^{24}$ +5.0° (*c*=0.9, $CHCl_3$). High-resolution positive-ion FAB-MS: Calcd for $C_{36}H_{36}NO_{12}$ $(M+H)^+$: 674.2238. Found: 674.2231. UV λ_{max}^{MeOH} nm (log ε): 269 (3.7). IR ($CHCl_3$): 1767, 1505, 1428, 1372, 1260, 1215, 1184, 1013 cm^{-1}. ^{13}C-NMR ($CDCl_3$, 125 MHz) δc: 20.58, 20.65 (Ac×2), 20.67 (Ac×4), 29.5 (5-C), 32.1 (13-C), 41.0 (α-C), 50.2 (14-C), 66.2 (8-C), 121.1 (1-C), 122.3 (9-C), 123.0 (5'-C), 123.3 (12-C), 123.6 (4-C), 124.4 (2'-C), 127.8 (6'-C), 132.1 (12a-C), 133.3 (4a-C), 135.5 (8a-C), 137.1 (14a-C), 138.8 (1'-C), 139.8, 139.9, 140.49, 140.54 (2-, 3-, 10-, 11-C), 140.3 (4'-C), 141.7 (3'-C), 168.3 (Ac×2), 168.42, 168.44, 168.50, 168.53 (Ac×4). FAB-MS: *m/z* 674 $(M+H)^+$, 632 $(M–Ac)^+$, 466 (M–diacetoxybenzyl) $^+$, 424 (M–diacetoxybenzyl –Ac) $^+$, 382 (M–diacetoxybenzyl–Ac×2) $^+$, 248 (M– $C_{23}H_{22}O_8$) $^+$.

14 The ^{1}H- and ^{13}C-NMR spectra of new compounds (**1**) and their derivatives (**1a**, **2a**) were assigned on the basis of homo- and heterocorrelation spectroscopy (^{1}H-^{1}H, ^{1}H-^{13}C COSY), and heteronuclear multiple bond connectivity (HMBC) experiments.

15 S. F. Dyke and R. G. Kinsman, 'In Heterocyclic Compounds, Isoquinolines,' Vol. 38, Part 1, ed. by G. Grethe, Wiley, New York, 1981, pp. 25–26.

16 E. B. Hanssen and H. C. Chiang, *J. Org. Chem.*, 1977, **42**, 3588.

17 D. Tourwe, G. Van Binst, and T. Kametani, *Org. Magn. Reson.*, 1977, **9**, 341.

18 K. Iwase and M. Cushman, *J. Org. Chem.*, 1982, **47**, 545.

19 **2a**: A white powder, $[\alpha]_D^{26}$ +8.0° (*c*=0.8, $CHCl_3$). High-resolution EI-MS: Calcd for $C_{36}H_{35}NO_{12}$ (M^+): 673.2159. Found: 673.2169. UV λ_{max}^{MeOH} nm (log ε): 268 (3.3). IR ($CHCl_3$): 1767, 1505, 1430, 1372, 1258, 1210, 1182, 1013 cm^{-1}. ^{13}C-NMR ($CDCl_3$, 125 MHz) δc: 20.5 (Ac), 20.67 (Ac×3), 20.70, 20.72 (Ac×2), 29.4 (5-C), 30.6 (13-C), 40.1 (α-C), 45.8 (6-C), 49.6 (14-C), 62.7 (8-C), 121.2 (1-C), 121.6 (11-C), 122.9 (5'-C), 123.6 (4-C), 124.0 (2'-C), 126.9 (12-C), 127.2 (6'-C), 131.4 (8a-C), 132.4 (12a-C), 133.2 (4a-C), 137.4 (14a-C), 139.4 (1'-C), 139.7, 139.9 (2-, 3-C), 140.30, 140.38, 140.41 (4'-, 9-, 10-C), 168.1, 168.3 (Ac), 168.4 (Ac×2), 168.53, 168.54 (Ac×2). EI-MS [20 eV, rel. int. (%)]: *m/z* 673 (M^+, 0.2), 631 (M^+–Ac, 0.2), 466 (M^+–diacetoxybenzyl, 47), 424 (M^+–diacetoxybenzyl–Ac, 100), 382 (M^+–diacetoxybenzyl–Ac×2, 73), 340 (M^+–diacetoxybenzyl–Ac×3, 22), 298 (M^+– diacetoxybenzyl–Ac×4, 6), 248 (M^+–$C_{23}H_{21}O_8$, 9).

20 M. D. Rosa, M. Radomski, R. Carnuccio, and S. Moncada, *Biochem. Biophys. Res. Commun.*, 1990, **172**, 1246.