Structures of Fructigenines A and B, New Alkaloids Isolated from *Penicillium fructigenum* TAKEUCHI

Kunizo ARAI,* Kazutake KIMURA, Taisei MUSHIRODA and Yuzuru YAMAMOTO

Faculty of Pharmaceutical Sciences, Kanazawa University, 13-1 Takara-machi, Kanazawa 920, Japan. *Received April 8, 1989*

Two alkaloids, fructigenines A (1) and B (2), have been isolated from *Penicillium fructigenum* TAKEUCHI and the structures were established on the basis of spectroscopic evidence and chemical transformations.

Keywords fructigenine A; fructigenine B; alkaloid; *Penicillium fructigenum*; hexahydropyrrolo[2,3-*b*]indole

In the course of our screening for biologically active substances among fungal metabolites, we isolated two new alkaloids from *Penicillium fructigenum* TAKEUCHI and named them fructigenines A (**1**) and B (**2**), respectively.

The fungus was stationarily cultured at 27 °C for 14 d in a modified Czapek–Dox medium (containing 5 g/l of polypeptone). The combined ethyl acetate extract of mycelia and culture broth was chromatographed on a column of silica gel. Elution with chloroform gave fructigenine B (**2**) as a colorless solid, and subsequent elution with chloroform–ethyl acetate (3:1 v/v) afforded fructigenine A (**1**) as a colorless solid.

Fructigenine A (**1**) was indicated to possess the molecular formula of $C_{27}H_{29}N_3O_3$ by the high resolution mass spectrum (HR-MS). It was positive to Dragendorff and van Urk's reagents. The ultraviolet (UV) spectrum of **1** showed absorptions at 246, 276 and 285 nm. The infrared (IR) spectrum of **1** showed an NH absorption at 3430 cm^{-1} and amide carbonyl absorptions at 1660 and 1605 cm^{-1}.

The presence of a 1,1-dimethyl-2-propenyl group in **1** was established by the proton nuclear magnetic resonance (^{1}H-NMR) spectrum (geminal methyl; λ 0.87 and 1.04 (each 3H, s), terminal olefin; δ 5.06 (1H, d, J=18 Hz), 5.10 (1H, d, J=10 Hz) and 5.78 (1H, dd, J=18, 10 Hz)) and the ^{13}C-NMR spectrum (δ 22.3 (q), 23.6 (q), 40.3 (s), 114.3 (t) and 142.9 (d)). A peak at m/z 374 (M^+-69) in the MS also supports the presence of this moiety.

Signals at δ_H 2.63 (3H, s), and δ_C 23.1 (q), 169.9 (s) in the ^{1}H- and ^{13}C-NMR spectra, and the fragmentation peak at m/z 401 (M^+-42) in the MS showed the presence of an acetyl group in the molecule.

The general appearance of the UV spectrum closely resembled that of the *N*-acetyl indoline chromophore.[1] Signals at δ 60.9 (s), 79.2 (d), 118.9 (d), 124.4 (d), 128.9 (d, C×2), 132.0 (s) and 143.3 (s) in the ^{13}C-NMR spectrum also indicated the presence of an indoline moiety. These chemical shifts are almost superimposable on the corresponding signals of LL-S490β,[1] roquefortine[2] and amauromine.[3] The presence of nine aromatic protons between δ 6.92—7.94 suggested the existence of another aromatic ring besides the benzene ring of the indoline moiety in **1**.

A strong IR absorption band at 1660 cm^{-1} and two signals at δ 164.6 and 167.9 in the ^{13}C-NMR spectrum indicate the presence of two amide functions. In the ^{1}H-NMR spectrum, two sets of ABX patterns due to two $-CH_2-CH-N-$ moieties appeared (δ 2.07 (1H, dd, J=12, 12.5 Hz), 2.52 (1H, dd, J=12.5, 5.5 Hz), 3.74 (1H, dd, J=12, 5.5 Hz) and 2.82 (1H, dd, J=14.5, 9.5 Hz), 3.44 (1H, dd, J=14.5, 3.5 Hz), 4.20 (1H, dd, J=9.5, 3.5 Hz)). These spectral data indicate the presence of a diketopiperazine system in the molecule of **1**.

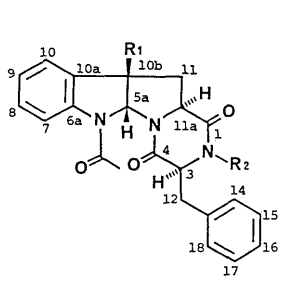
Hydrolysis of **1** with 6 N HCl at 110 °C for 5 h yielded L-tryptophan and L-phenylalanine as degradation products. Thus, the structure of **1** was supposed to be a hexahydropyrrolo[2,3-*b*]indole derivative, consisting of tryptophan and phenylalanine.

The placement of the acetyl group at N-1 was indicated by the fact that signals of the 5a-methine proton (δ 6.04) and 7-aromatic proton (δ 7.94) were recognized at lower field than those of the corresponding protons in compounds possessing the same skeleton, such as roquefortine (methine, δ 5.70; aromatic protons, δ 6.53—7.30), amauromine (δ 5.42; 6.46—7.16) and ditryptophenaline (δ 4.89; 6.5—7.7).[4] Treatment of **1** with acetic anhydride under reflux for 4 h gave an *N*-monoacetate (**1a**) at the NH group of the diketopiperazine moiety.[5]

The placement of a reversed isoprene unit at C-10b in the indoline moiety was determined from the singlet signal of C-10b (δ 60.9) in the ^{13}C-NMR spectrum which was assignable by comparison with those of related compounds, such as roquefortine (δ 61.5 (s)), aszonalenin[5] (δ 61.1 (s)) and amauromine (δ 61.8 (s)).

From the above results, the plane structure of fructigenine A was deduced to be **1**.

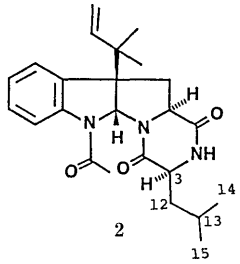
This structure was also supported strongly by the MS fragmentation pattern.[6] Namely, the primary fragmentation of **1** involving loss of an acetyl radical (42 mass units) gave a peak at m/z 401 and the subsequent loss of a C_5H_9 radical gave a prominent fragment peak (fragment A) at



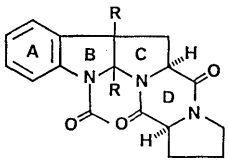
1 : R_1 =  , R_2=H

1a : R_1 =  , R_2= acetyl

1b : R_1 =  , R_2=H



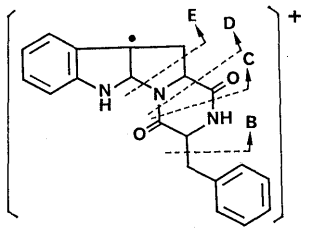
2



3 : R= H

4 : R= H

Chart 1



fragment A

Chart 2

m/z 332 (base peak). Fragments B (*m/z* 241) and E (*m/z* 130) were reasonable as the peaks originated from cleavage in the expected positions alpha to the diketopiperazine ring of fragment A, respectively, and fragments C (*m/z* 185) and D (*m/z* 157) originated from cleavage of the diketopiperazine ring of fragment A were also observed.

The stereochemistry of fructigenine A including the absolute configuration was established as follows. A nuclear Overhauser effect (NOE) enhancement (24%) of the H-5a signal upon irradiation of the methyl proton signal of the isoprenyl group showed the *cis*-fusion of the B and C rings. The fact that L-tryptophan and L-phenylalanine were isolated by acid hydrolysis of **1** proved the absolute configurations at C-3 and C-11a to be both *S*. Further, in a comparison of the circular dichroism (CD) spectral data of dihydro-fructigenine A (**1b**) (+27400 (247 nm)) with those of related compounds (**3**; +60200 (245.5 nm), **4**; −82300 (236 nm))[3] which have the same ring system as that of fructigenine A, its positive sign suggests that **1** has the same type of absolute configuration as that of **3** at the B and C ring junction.

Fructigenine B (**2**), $C_{24}H_{31}N_3O_3$ (by HR-MS), was positive to Dragendorff and van Urk's reagents. The UV spectrum of **2** was similar to that of **1**. The UV spectrum of **2** (247, 277 and 285 nm) showed the presence of an indoline chromophore and the absorption bands at 3450 and 1675 cm^{-1} in the IR spectrum indicated the existence of a diketopiperazine moiety. A prominent peak at *m/z* 130 was assigned to the indoline-3-methylene ion. In the ^{1}H-NMR spectrum, the presence of 1,1-dimethyl-2-propenyl and acetyl groups was recognized and four aromatic protons which were assignable to benzene ring protons of an indoline moiety were observed. But, signals due to the phenylalanine moiety in **1** was not observed, while new signals of two methyl groups (δ_H 0.98, 0.99 (each 3H, d, J = 5 Hz); δ_C 21.1 (q), 21.2 (q)) and one methine (δ_C 24.4 (d)) were seen in the NMR spectrum. These data suggested that the phenylalanine moiety in **1** has been replaced by a leucine moiety in **2**. This was supported by acid hydrolysis of **2** to afford L-leucine and L-tryptophan. Further, the CD spectrum of dihydro-**2** (+25100 (248 nm)) was similar to that of **3**.

From these results, the structure of **2** was determined and its absolute configuration was established to be the same as that of **1**.

Fructigenine A (**1**) has a growth inhibitory activity against *Avena coleoptile*[7] and L-5178Y cells (unpublished result).

Experimental

Melting points were taken using a Yanagimoto micro melting point apparatus and are uncorrected. UV spectra were measured on a Hitachi 323 spectrometer. IR spectra were measured on a JASCO A-102 infrared spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL FX-100. The chemical shifts are given in ppm (δ) relative to internal tetramethylsilane (TMS), coupling constants (J) are in Hz and multiplicities are indicated by the usual symbols. Optical rotations were determined on a JASCO DIP-140 polarimeter. CD curves were recorded on a JASCO J-20 spectropolarimeter. MS and HR-MS were measured on a Hitachi M-80 mass spectrometer. Column chromatography was performed on Wakogel C-200. Thin layer chromatography (TLC) was carried out on Kieselgel 60PF 254 for analytical TLC unless otherwise noted. Preparative TLC (PLC) was carried out using Silica gel 60F 254 plates (Merck). Malt extract, polypeptone and glucose were purchased from Difco Co., Daigoeiyo Co., and Daiichi Seiyaku Co., respectively.

TABLE I. ^{13}C-NMR Spectral Data of Fructigenines A and B[a]

Position	Fructigenine A (**1**)	Fructigenine B (**2**)
1	164.6 (s)	165.9 (s)
3	55.9 (d)	53.2 (d)
4	167.9 (s)	169.0 (s)
5a	79.2 (d)	79.4 (d)
6a	143.3 (s)	143.2 (s)
7	124.4 (d)	124.4 (d)
8	128.9 (d)	128.9 (d)
9	118.9 (d)	119.1 (d)
10	128.9 (d)	128.9 (d)
10a	132.0 (s)	132.2 (s)
10b	60.9 (s)	60.8 (s)
11	37.0 (t)	38.9 (t)
11a	59.0 (d)	59.0 (d)
12	36.2 (t)	35.8 (t)
13	135.3 (s)	24.4 (d)
14	129.1 (d)	21.1 (q)
15	129.2 (d)	21.2 (q)
16	127.5 (d)	—
17	129.2 (d)	—
18	129.1 (d)	—
Acetyl	169.9 (s), 23.1 (q)	170.0 (s), 23.2 (q)
Isoprene	40.3 (s), 142.9 (d), 114.3 (t), 23.3 (q), 23.6 (q)	40.3 (s), 143.0 (d), 114.5 (t), 22.3 (q), 23.5 (q)

a) $CDCl_3$ solution, δ in ppm from TMS internal standard.

Isolation of Fructigenines A and B *Penicillium fructigenum* TAKEUCHI was cultivated at 27 °C for 14 d in 30 Roux flasks each containing 200 ml of medium composed of sucrose 15 g, $NaNO_3$ 3 g, $MgSO_4$ 0.45 g, KCl 0.5 g, $FeSO_4$ 0.01 g, K_2HPO_4 1 g, polypeptone 0.5 g, and distilled water 1 l. The culture broth was concentrated to one-fourth of the initial volume and extracted with ethyl acetate. The ground mycelia were also extracted with ethyl acetate. The organic layers were combined, dried and concentrated to dryness. The residue was subjected to silica gel chromatography using chloroform and a mixed solvent of chloroform–ethyl acetate successively. From the fraction eluted with chloroform, methyl 4-[2-(2*R*)-hydroxyl-3-butynyloxy]benzoate[8] (2 mg) and fructigenine B (25 mg) were obtained. A mixture of fructigenine A and cyclopenin, and then aspterric acid[8] (120 mg) were eluted with 10% ethyl acetate–chloroform. The mixed fraction was separated by PLC (chloroform–ethyl acetate (3 : 1 v/v)) to give cyclopenin[9] (14 mg) from the lower band. The crude fructigenine A obtained from the upper band was purified by alumina chromatography with ethyl acetate to afford the pure metabolite (160 mg). Cyclopenol[8] (42 mg) was obtained from the 30% ethyl acetate–chloroform eluate.

Fructigenine A (1) HR-MS *m/z*: 443.2200 (calcd for $C_{27}H_{29}N_3O_3$, 443.2206), 401.2045 ($C_{25}H_{27}N_3O_2$, 401.1118), 374.1075 ($C_{22}H_{20}N_3O_3$, 374.1075), 332.1441 ($C_{20}H_{18}N_3O_2$, 332.1398), 241.0851 ($C_{13}H_{11}N_3O_2$, 241.0851), 185.0809 ($C_{11}H_9N_2$, 185.0714), 157.0816 ($C_{10}H_9N$, 157.0816), 130.0681 (C_9H_8N, 130.0657). EI-MS *m/z*: 443, 401, 374, 332, 241, 185, 157, 130, 69. UV (EtOH) nm (log ε): 246 (4.10), 276 (3.35), 285 (3.27). IR (KBr) cm^{-1}: 3430, 1690 (sh), 1660, 1605. ^{1}H-NMR ($CDCl_3$) δ: 0.87, 1.04 (each 3H, s), 2.07 (1H, dd, J = 12, 12.5 Hz), 2.52 (1H, dd, J = 12.5, 5.5 Hz), 2.63

(3H, s), 2.82 (1H, dd, $J=14.5$, 9.5 Hz), 3.44 (1H, dd, $J=14.5$, 3.5 Hz), 3.74 (1H, dd, $J=12$, 5.5 Hz), 4.20 (1H, dd, $J=9.5$, 3.5 Hz), 5.06 (1H, d, $J=$ 18 Hz), 5.10 (1H, d, $J=10$ Hz), 5.78 (1H, dd, $J=18$, 10 Hz), 5.94 (1H, br s, exchangeable with D_2O), 6.04 (1H, br s),-6.92—7.40 (8H, m), 7.94 (1H, dd, $J=7.5$, 1.5 Hz), $[\alpha]_D$ −178° ($c=0.24$, $CHCl_3$).

Acetylfructigenine A (1a) Acetic anhydride (2 ml) was added to a solution of fructigenine A (50 mg) in pyridine (2 ml) and the mixture was refluxed for 4 h. The cooled reaction mixture was concentrated to dryness *in vacuo* to afford an oil which was subjected to PLC (chloroform–ethyl acetate (9 : 1 v/v)) to give a monoacetate (**1a**) as colorless needles, (32 mg), mp 196—198 °C, from ether–hexane. *Anal.* Calcd for $C_{29}H_{31}N_3O_4$: C, 71.73; H, 6.44; N, 8.65. Found: C, 71.47; H, 6.45; N, 8.63. MS *m/z*: 485 (M^+), 443, 372, 332, 241, 185, 157, 130, 69. UV (EtOH) nm (log ε): 245.5 (4.13), 277 (3.51), 286 (3.45). IR (KBr) cm^{-1}: 1705, 1675 (sh), 1664, 1650 (sh). ^{1}H-NMR ($CDCl_3$) δ: 0.92, 0.98 (each s, 3H), 1.00 (1H, m), 2.12 (1H, dd, $J=4.5$, 12 Hz), 3.32 (1H, dd, $J=13.5$, 5 Hz), 3.48 (1H, dd, $J=13.5$, 3.5 Hz), 3.76 (1H, dd, $J=12$, 4.5 Hz), 5.04 (1H, d, $J=10.5$ Hz), 5.10 (1H, d, $J=16.5$ Hz), 5.22 (1H, dd, $J=4.5$, 3.5 Hz), 5.60 (1H, dd, $J=16.5$, 10 Hz), 6.11 (1H, s), 6.90—7.40 (8H, m), 8.00 (1H, d, $J=8$ Hz).

Dihydrofructigenine A (1b) A solution of fructigenine A (50 mg) in ethyl acetate (20 ml) was hydrogenated over 5% palladium carbon (50 mg) at room temperature for 1 h. The catalyst was filtered off and the filtrate was concentrated to dryness. The residue was purified by PLC (chloroform–ethyl acetate (9 : 1 v/v)) to give a dihydro derivative (**1b**) (46 mg) as an amorphous solid. MS *m/z*: 445 (M^+), 403, 332, 241, 185, 157, 130. UV (EtOH) nm (log ε): 247 (4.06), 277 (3.32), 285.5 (3.24). IR (KBr) cm^{-1}: 3450, 1685 (sh), 1665, 1645 (sh). ^{1}H-NMR ($CDCl_3$) δ: 0.84 (3H, d, $J=7$ Hz), 0.92 (6H, s), 1.22 (2H, q, $J=7$ Hz), 2.12 (1H, t, $J=12$ Hz), 2.52 (1H, dd, $J=12$, 5 Hz), 2.70 (3H, s), 2.90 (1H, dd, $J=14.5$, 8.5 Hz), 3.52 (1H, dd, $J=14.5$, 4 Hz), 3.76 (1H, dd, $J=12$, 5 Hz), 4.28 (1H, dd, $J=9.5$, 4 Hz), 6.06 (1H, s), 6.38 (1H, s), 6.95—7.40 (8H, m), 7.95 (1H, d, $J=$ 7.5 Hz). CD (EtOH) [θ] (nm): +27400 (247).

Hydrolysis of Fructigenine A (1) A suspension of fructigenine A (300 mg) in 6 N HCl was heated at 110 °C for 4 h under a nitrogen atmosphere. The cooled reaction mixture was concentrated to dryness *in vacuo* and the residue was extracted with water. The extracted solution was evaporated to dryness and the residue was purified by cellulose TLC (*n*-butanol–acetic acid–water (60 : 15 : 25 v/v)) and separated into two ninhydrin positive bands. L-Phenylalanine, $[\alpha]_D$ −4.1° ($c=0.1$, 0.1 N HCl) (7 mg), was obtained from the upper band, and L-tryptophan, $[\alpha]_D$ +4.5° ($c=0.05$, 0.1 N HCl) (1 mg), was isolated from the lower band. Each product was identical with an authentic sample in the *Rf* value on TLC and the optical rotation.

Fructigenine B (2) HR-MS *m/z*: 409.2366 (required $C_{24}H_{31}N_3O_3$, 409.2363), 367.2220 ($C_{22}H_{29}N_3O_3$, 367.2257), 298.1533 ($C_{17}H_{20}N_3O_2$, 298.1535), 185.0732 ($C_{11}H_{19}N_2O$, 185.0715), 130.0657 (C_9H_8N, 130.0656), 86.0983 ($C_5H_{12}N$, 86.0969). EI-MS *m/z*: 409 (M^+), 367, 298, 270, 185, 157, 130, 86. UV (EtOH) nm (log ε): 247 (3.98), 277 (3.20), 285 (3.18). IR (KBr) cm^{-1}: 3450, 2960, 1690 (sh), 1675, 1660 (sh). ^{1}H-NMR ($CDCl_3$) δ: 0.97, 0.98 (each 3H, d, $J=5$ Hz), 1.04, 1.20 (each 3H, s), 1.35—2.28 (3H, m), 2.54 (1H, dd, $J=11.5$, 8.5 Hz), 2.68 (3H, s), 3.72—4.02 (3H, m), 5.12 (1H, d, $J=18$ Hz), 5.16 (1H, d, $J=10$ Hz), 5.84 (1H, dd, $J=18$, 10 Hz), 6.06 (1H, s), 6.32 (1H, s, exchangeable with D_2O), 7.04—7.44 (3H, m), 8.00 (1H, br d, $J=7.5$ Hz). $[\alpha]_D$ −161.1° ($c=0.47$, $CHCl_3$).

Hydrogenation of Fructigenine B A solution of fructigenine B (50 mg) in ethyl acetate (20 ml) was hydrogenated according to the procedure described for fructigenine A. Dihydro-fructigenine B was obtained as an amorphous solid (45 mg). MS *m/z*: 411 (M^+), 369, 298, 270, 185, 157, 130, 86. UV (EtOH) nm (log ε): 248 (3.90), 277 (3.07), 285 (3.01). IR (KBr) cm^{-1}: 1675. ^{1}H-NMR ($CDCl_3$) δ: 0.78 (3H, t, $J=7.5$ Hz), 0.80—1.02 (12H, m, Me × 4), 1.44 (2H, q, $J=7.5$ Hz), 1.40—2.32 (3H, m), 2.50 (1H, dd, $J=12$, 8.5 Hz), 2.60 (3H, s), 3.64—4.16 (3H, m), 6.08 (2H, br s, NH and methine), 6.95—7.56 (3H, m), 7.92 (1H, d, $J=7.5$ Hz). CD [θ] (nm): +25100 (248).

Acid Hydrolysis of Fructigenine B Fructigenine B (400 mg) was hydrolyzed according to the same procedure as described for fructigenine A. The hydrolyzed mixture was separated by cellulose TLC to give L-leucine $[\alpha]_D$ +17.5° ($c=0.05$, 0.1 N HCl) and L-tryptophan $[\alpha]_D$ +3.9° ($c=0.04$, 0.1 N HCl).

Acknowledgment We are grateful Dr. M. Ohkawa, Kanazawa University, for providing the fungus. We also wish to thank Mr. Y. Itatani and Misses I. Okunaka, E. Oda, in our Faculty, for elemental analysis and spectral measurements.

References and Notes

1) G. A. Ellestad, P. Mirando and M. P. Kunstmann, *J. Org. Chem.*, **38**, 4204 (1973).
2) P. M. Scott, M. Merrien and J. Polonsky, *Experientia*, **32**, 140 (1976).
3) S. Takase, Y. Kawai, I. Uchida, H. Tanaka and H. Aoki, *Tetrahedron*, **41**, 3037 (1985).
4) J. P. Springer, G. Büchi and J. Clardy, *Tetrahedron Lett.*, **1977**, 2403.
5) Y. Kimura, T. Hamasaki, H. Nakajima and A. Isogai, *Tetrahedron Lett.*, **23**, 225 (1982).
6) S. Ohmomo, K. Ogawa, T. Ohashi and M. Abe, *Agric. Biol. Chem.*, **42**, 2387 (1978).
7) The *Avena coleoptile* growth test of **1** was carried out by Dr. Ohkawa, Department of Education, Kanazawa University.
8) T. Mushiroda, H. Miyajima, K. Arai, Y. Yamamoto, M. Uneme and M. Numata, in preparation. Properties of methyl 4-[2-(2*R*)-hydroxyl-3-butynyloxy]benzoate: mp 111—112°C, Molecular formula: $C_{12}H_{12}O_4$. MS *m/z*: 220, 189, 165, 153, 135, 121. ^{1}H-NMR ($CDCl_3$) δ: 2.55 (1H, d, $J=2.2$ Hz), 2.76 (1H, br s), 3.89 (3H, s), 4.16 (1H, d, $J=4.5$ Hz), 4.79 (1H, ddd, $J=6.0$, 4.5, 2.2 Hz), 6.94 and 7.99 (each 2H, d, $J=9.0$ Hz). ^{13}C-NMR ($CDCl_3$) δ: 51.9 (q), 61.1 (d), 71.4 (t), 74.6 (d), 80.8 (s), 114.3 (d, C × 2), 123.3 (s), 131.6 (d, C × 2), 161.8 (s), 166.7 (s). $[\alpha]_D$ −12.3 ($c=0.1$, $CHCl_3$). IR (KBr) cm^{-1}: 3455, 3229, 2949, 2091, 1684.
9) J. H. Birkinshaw, M. Luckner, Y. S. Mohammed, K. Mothes and C. E. Stickings, *Biochem. J.*, **89**, 196 (1963); M. Luckner, *European J. Biochem.*, **2**, 74 (1967).