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Studies on the Constituents of the Stems of *Tinospora tuberculata* BEUMÉE. I. *N-trans*- and *N-cis*-Feruloyl Tyramine, and a New Phenolic Glucoside, Tinotuberide

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From the dried stems of *Tinospora tuberculata* BEUMÉE (Menispermaceae), *N-trans*-feruloyl tyramine (**1**), *N-cis*-feruloyl tyramine (**2**), and a new phenolic glucoside, tinotuberide (**6**), were isolated. The structure of **6** was elucidated as 3-(4′-β-D-glucopyranosyloxy-3′,5′-dimethoxyphenylmethoxy)-2-*trans*-propen-1-ol.

Keywords——Borapet; *Tinospora tuberculata;* Menispermaceae; *N-trans*-feruloyl tyramine; *N-cis*-feruloyl tyramine; tinotuberide; 3-(4′-β-D-glucopyranosyloxy-3′,5′-dimethoxyphenylmethoxy)-2-*trans*-propen-1-ol

The stem of *Tinospora tuberculata* BEUMÉE (syn. *T. crispa* DIERS; Thai name, Borapet; Menispermaceae)[1] is one of the most popular traditional drugs in Thailand and other South-east Asian countries, and is used as an appetizer, as a febrifuge for malaria and smallpox, and as a remedy for many other purposes. It is said to make the blood "bitter and cool" in the terms of Thai old-style medicine.[2]

In 1906, Bacon[3] reported the presence of a glycosidic bitter principle. Two alkaloids, named tinosporine and tinosporidine,[4] picroretin as its bitter principle,[5] waxes, hydrocarbons and a phytosterol[6] were also recorded in other papers.

In this paper, we report the identification of two phenolic acid amides, and the structure elucidation of a new phenolic glucoside, tinotuberide. The methanolic extract of the crude drug "Borapet" (the stems of this plant), obtained in Bangkok, Thailand,[7] was dissolved in water, and extracted successively with hexane, ethyl acetate and butanol. Examinations and

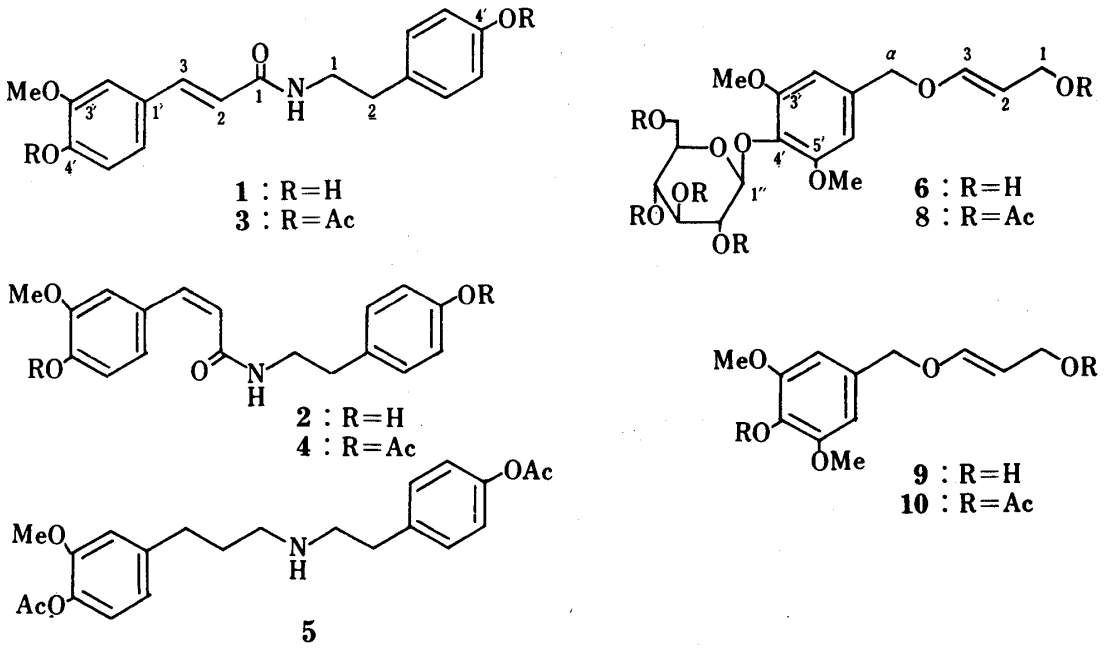

Chart 1

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repeated purifications of the ethyl acetate fraction gave compound **1**, as colorless plates, and compound **2**, as a pale yellow oil.

Compound **1**, $C_{18}H_{19}O_4N$, mp 91°C, gave a diacetate (**3**), colorless needles, $C_{22}H_{23}O_6N$, mp 157°C, on acetylation, and tyramine (*p*-hydroxyphenethyl amine) as its hydrochloride, mp 240—242°C, on hydrolysis with hydrogen chloride in ethanol. Physical data derived from the infrared (IR) spectrum, ultraviolet (UV) spectrum, ^{1}H-nuclear magnetic resonance (NMR) spectrum, ^{13}C-NMR spectrum (Table I) and mass spectrum (MS) suggested that this compound was an acid amide of *trans*-ferulic acid and tyramine. By direct comparison of its diacetate (**3**) with an authentic sample,[8] compound **1** was identified as *N-trans*-feruloyl tyramine (**1**).

Compound **2**, MS *m/z*: 313 (M^+) showed very similar mass fragmentation to **1**, and gave the same dihydrodiacetate (**5**) as derived from **1** in the same manner, on hydrogenation followed by acetylation. In the ^{1}H-NMR, the coupling constant (*J* value in Hz) of the pair of doublet signals corresponding to the olefinic protons was reduced from 15.5 Hz in **1** (δ 7.37 and 6.45) to 12.5 Hz in **2** (δ 6.44 and 5.68). A similar difference was also observed between their diacetates, **3** and **4**. On UV irradiation (high pressure mercury lamp), pure samples of **1** and **2** were both converted to a mixture of **1** and **2**, having the same ratio (6:10). From these experiments, the structure of **2** was elucidaed as *N-cis*-feruloyl tyramine (**2**). This is the first

TABLE I. ^{13}C-NMR Data for *N-trans*- (**1**) and *N-cis*-Feruloyl Tyramine (**2**) and Related Compounds (δ)

Carbon	Multiplicity	**1**[a]	**2**[a]	**3**[b]	**4**[b]	**5**[b]	Ferulic acid[a]	Tyramine[a]
Feruloyl								
1	s	165.1	166.2	165.5	166.9	169.0	167.8	
2	d	138.6	136.7	139.5	139.7	38.2t	144.3	
3	d	110.4	114.2	111.1	113.3	31.5t	111.1	
1′	s	147.8	147.3	148.8	149.2	149.2	149.0	
2′	d	118.6[c]	120.9[c]	121.0[c]	122.6[c]	120.3[c]	115.5	
3′	s	126.1	126.7	133.6[d]	133.9[d]	136.4[d]	125.7	
4′	s	147.4	146.8	140.4	140.0	139.9	147.8	
5′	d	115.3	114.8	120.1	122.0	112.7	115.0	
6′	d	121.2[c]	124.2[c]	122.8[c]	124.6[c]	122.5[c]	122.6	
CH_3O	q	55.4	55.5	55.7	55.9	55.8	55.6	
Tyramine								
1	t	40.5	40.6	40.7	40.4	40.5		40.3
2	t	34.3	34.2	34.8	34.6	35.0		32.0
1′	s	129.1	129.3	136.3[d]	135.8[d]	138.1[d]		127.2
2′,6′	d	129.1	129.3	129.4	129.5	129.6		129.3
3′,5′	d	114.8	115.1	121.3	121.6	121.5		108.4
4′	s	155.2	155.5	150.9	150.8	150.9		156.0
Acetoxy								
CH_3	q			20.5 21.0	20.5 21.0	20.5 21.0		
CO	s			168.4 169.3	168.8 169.5	169.5 171.9		

a) Measured in DMSO-d_6 at 22.5 MHz.
b) Measured in $CDCl_3$ at 22.5 MH_Z.
c, *d*) Assignments may be reversed in each column.

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TABLE II. ^{1}H-NMR Data for Tinotuberide (**6**) and Related Compounds (δ)

Hydrogen	**6**[a]	**8**[b]	**9**[a]	**10**[b]	**7**[a]
1	4.08 (2H, d, J=5)	4.72 (2H, d, J=6)	4.08 (2H, d, J=6)	4.72 (2H, d, J=6)	1.5—1.9[c] (2H, m)
2	6.1—6.6[c] (1H, m)	6.19 (1H, dt, J=6, 16)	6.16 (1H, dt, J=6, 16)	6.25 (1H, dt, J=6, 16)	1.5—1.9[c] (2H, m)
3	6.1—6.6[c] (1H, m)	6.59 (1H, d, J=16)	6.43 (1H, d, J=16)	6.57 (1H, d, J=16)	4.2—4.6[d] (2H, m)
2′,6′	6.71 (2H, s)	6.61 (2H, s)	6.65 (2H, s)	6.63 (2H, s)	6.50 (2H, s)
CH_3O-,α	3.77 (8H, s)	3.84 (8H, s)	3.76 (8H, s)	3.83 (8H, s)	3.73 (8H, s)
1″	4.10 (1H, d, J=7)	4.20 (1H, d, J=7)			4.2—4.6[d] (1H, m)
CH_3CO-		2.03s, 2.04s 2.10s (15H)		2.10s, 2.33s (6H)	

a) Measured in DMSO-d_6 at 90 MHz.
b) Measured in $CDCl_3$ at 90 MHz.
c, *d*) Overlapping signals in each column.

report of its natural occurrence.

The butanol fraction was successively subjected to droplet counter current chromatography (DCCC) and silicic acid column chromatgraphy, and a new phenolic glucoside, named tinotuberide (**6**), colorless needles, $C_{18}H_{26}O_{10}$, mp 194—195°C, $[\alpha]_D^{18}$ −15.6° (MeOH), was obtained. Tinotuberide (**6**) gave a dihydro compound (**7**) on hydrogenation, and a penta-acetate (**8**), $C_{28}H_{36}O_{15}$, mp 110.5—111.5°C, $[\alpha]_D^{25}$ −14.2° ($CHCl_3$), on acetylation with acetic anhydride in pyridine.

On enzymatic hydrolysis of **6** with β-D-glucosidase, an unstable phenolic alcohol (**9**) and D-glucose, $[\alpha]_D^{25}$ +50°, were obtained. In the ^{1}H-NMR (Table II) of **6** and **8**, the doublet signals assigned to their anomeric protons at δ 4.10 and 4.20, respectively, showed a J-value of 7 Hz. These results suggested existence of a β-D-glucopyranosyl moiety in the structure of **6**.

The phenolic alcohol (**9**) showed ^{1}H-NMR signals attributable to two olefinic protons on a *trans* double bond at δ 6.43 (d, J=16 Hz) and δ 6.16 (dt, J=6, 16 Hz), and a carbinol methylene adjacent to the double bond at δ 4.08 (d, J=6 Hz). The signal of this carbinol methylene was found to be shifted to δ 4.72 in its diacetate (**10**). As this acetylation shift was also observed between **6** and **8** (Table II), a partial structure, 3-*O*-substituted 2-*trans*-propen-1-ol, in **6** as well as in **9** was deduced.

The ^{1}H-NMR (Table II) and ^{13}C-NMR(Table III) of tinotuberide (**6**) and its derivatives suggested the remainder of the structure to be a symmetrically substituted aromatic ring having an axis including an *O*-function and an *O*-substituted methylene, bearing two methoxyl groups. On hydrolysis of **6**, remarkable shifts of the ^{13}C-NMR signals were observed at δ 132.7 (δ 129.2 in **9**) and δ 152.8 (δ 148.0 in **9**) which were attributable to a carbon (C-4′) bearing a glucoxy group and two carbons (C-3′ and 5′) bearing a methoxyl group, respectively, and no significant difference was observed in any other carbon of the aromatic ring or the *O*-substituted methylene group. These observations suggested a 4-glucoxy-3,5-dimethoxyphenylmethoxyl partial structure in **6**.

Thus, the structure of tinotuberide was elucidated as 3-(4′-β-D-glucopyranosyloxy-3′,5′-dimethoxyphenylmethoxy)-2-*trans*-propen-1-ol (**6**).

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TABLE III. ^{13}C-NMR Data for Tinotuberide (**6**) and Related Compounds (δ)

Carbon	Multiplicity	**6**[a]	**8**[b]	**9**[a]	**10**[b]	**7**[a]
1	t	61.6	64.8	61.7	64.9	34.1
2	d	128.5	123.3	127.4	123.7	31.8t
3	d	130.2	133.9	127.8	133.9	60.1t
1′	s	134.1	134.6	135.4	134.6	137.8
2′,6′	d	104.6	104.3	103.9	103.5	106.5
3′,5′	s	152.8	153.1	148.0	152.0	152.4
4′	s	132.7	133.1	129.2	133.0	132.6
α CH_3O-	t, q	56.5	56.4	56.0	56.2	56.3
1″	d	102.8	101.1			102.8
2″	d	74.3	72.1			74.1
3″	d	77.2	73.2			77.0
4″	d	70.1	68.7			69.9
5″	d	76.6	72.1			76.4
6″	t	62.3	62.4			60.9
CH_3CO-	q		20.6, 20.9		20.4, 21.0	
	s		169.3, 170.3, 170.5, 170.7		168.3, 168.5	

a) Measured in DMSO-d_6 at 22.5 MHz.
b) Measured in $CDCl_3$ at 22.5 MHz.

Experimental

All melting points were taken on a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were measured with a Hitachi EPI-G3 or a Shimadzu IR-408 spectrometer. UV, NMR and mass spectra were recorded using a Hitachi 200-20 spectrometer, a JEOL FX-90Q FT-NMR spectrometer (chemical shifts are expressed in δ value (ppm) using tetramethylsilane as an internal standard) and a JEOL JMS-D100 mass spectrometer, respectively. High-performance liquid chromatography (HPLC) were performed on a Waters 204A liquid chromatograph equipped with a UV detector (254 nm).

Isolation of *N-trans*-Feruloyl Tyramine (1) and *N-cis*-Feruloyl Tyramine (2)——The crude drugs (20 kg; dried stems of *Tinospora tuberculata* BEUMÉE, collected in Bangkok[7]) were powdered and extracted with MeOH under reflux. After concentration *in vacuo*, the MeOH extract (1790 g) was dissolved in H_2O, and the solution was extracted successively with hexane, EtOAc and butanol. The EtOAc fraction was chromatographed over a silicic acid column and eluted with $CHCl_3$/MeOH/H_2O (30:1.6:0.1) to give a crystalline mixture. This was recrystallized to give **1**, and the mother liquor was separated to provide **1** (8.3 g, 0.042% in total) and **2** (0.6 g, 0.003%) by HPLC (Waters Radialpak A, H_2O/MeOH, 55:45).

***N-trans*-Feruloyl Tyramine (1)**——Colorless plates (from $CHCl_3$/MeOH), mp 91°C. *Anal.* Calcd for $C_{18}H_{19}NO_4 \cdot CHCl_3$: C, 52.73; H, 4.66; N, 3.24. Found: C, 52.96; H, 4.47; N, 3.40. IR ν_{max}^{Nujol} cm^{-1}: 3200, 1655, 1548. UV λ_{max}^{EtOH} nm(ϵ): 285 (11900), 293 (11900), 317 (13300). ^{1}H-NMR (DMSO-d_6) δ: 3.81 (3H, s), 6.45 (1H, d, J=15.5 Hz), 6.61—7.24 (7H, m), 7.37 (1H, d, J=15.5 Hz), 7.98 (1H, t, J=6.0 Hz). ^{13}C-NMR: Table I. MS *m/z*: 313 (M^+), 193, 192, 177, 145, 120.

Acetylation of **1** with acetic anhydride in pyridine, gave *N-trans*-feruloyl tyramine diacetate (**3**), colorless needles (from hexane/EtOAc), mp 157—157.5°C. *Anal.* Calcd for $C_{22}H_{23}NO_6$: C, 66.49; H, 5.83; N, 3.52. Found: C, 66.43; H, 5.82; N, 3.43. IR ν_{max}^{Nujol} cm^{-1}: 1770, 1658, 1560. ^{1}H-NMR ($CDCl_3$) δ: 2.26 (3H, s), 2.28 (3H, s), 2.80 (2H, t, J=6.0 Hz), 3.61 (2H, q, J=6.0 Hz), 3.75 (3H, s), 6.30 (1H, d, J=15.5 Hz), 6.34 (1H, t, J=6.0 Hz), 6.86—7.52 (7H, m),7.52 (1H, d, J=15.5 Hz). ^{13}C-NMR: Table I. MS *m/z*: 397 (M^+), 355, 192, 177, 120. This product was identical with an authentic sample.[8]

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Hydrogenation of **1** (100 mg) by an ordinary procedure over Pd–C in MeOH, followed by acetylation with acetic anhydride in pyridine, gave *N*-dihydroferuloyl tyramine diacetate (**5**; 53 mg), colorless needles (from hexane/EtOAc), mp 121—122°C. *Anal.* Calcd for $C_{22}H_{25}NO_6$: C, 66.15; H, 6.31; N, 3.51. Found: C, 66.10; H, 6.33; N, 3.60. IR $\nu_{max}^{Nujol}cm^{-1}$: 3275, 1755, 1640, 1545. ^{1}H-NMR ($CDCl_3$) δ:2.27 (6H, s), 2.37 (2H, t, J=8.0 Hz), 2.72 (2H, t, J=8.0 Hz), 2.89 (2H, t, J=6.0 Hz), 3.40 (2H, q, J=6.0 Hz), 3.76 (3H, s), 5.74 (1H, t, J=6.0 Hz), 6.6—7.4 (7H, m). ^{13}C-NMR: Table I. MS m/z: 399 (M^+), 357, 195, 179, 150, 137, 120, 107.

Irradiation of **1** in MeOH with a 400 W high pressure mercury lamp for 5 h gave a mixture of **1** and **2** in the ratio of 6:10 (HPLC).

Tyramine Hydrochloride———On reflux with 5% HCl/EtOH (5 ml) for 20 h, **1** (100 mg) gave 15 mg of tyramine hydrochloride, colorless needles, mp 240—242°C. IR $\nu_{max}^{KBr}cm^{-1}$: 3400, 3100, 1610. MS m/z: 137 (M^+), 108, 107, 91, 77. This product was identical with a commercial sample of tyramine hydrochloride.

***N-cis* Feruloyl Tyramine (2)**———Pale yellow oil. IR $\nu_{max}^{film}cm^{-1}$: 3400—3100, 1645, 1585. ^{1}H-NMR (DMSO-d_6) δ: 3.76 (3H, s), 5.68 (1H, d, J=12.5 Hz), 6.44 (1H, d, J=12.5 Hz), 6.6—7.7 (7H, m), 8.09 (1H, t, J=6.0 Hz). ^{13}C-NMR: Table I. MS m/z: 313 (M^+), 193, 192, 177, 145, 120, 108, 107.

Acetylatin of **2** with acetic anhydride in pyridine gave *N-cis*-feruloyl tyramine diacetate (**4**) as an oil. IR $\nu_{max}^{film}cm^{-1}$: 1750, 1650, 1200. ^{1}H-NMR ($CDCl_3$) δ: 2.25, 2.28 (each 3H, s), 2.70 (2H, t, J=6.0 Hz), 3.44 (2H, q, J=6.0 Hz), 3.78 (3H, s), 5.87 (1H, d, J=12.5 Hz), 6.09 (1H, t, J=6.0 Hz), 6.60 (1H, d, J=12.5 Hz), 6.2—7.6 (7H, m). ^{13}C-NMR: Table I.

Hydrogenation and acetylation of **2** (100 mg) by the same procedure as **1**, gave *N*-dihydroferuloyl tyramine diacetate (**5**; 66 mg). This was identical with **5** derived from **1**.

UV irradiation of **2** in the same manner as **1** gave a mixture of **1** and **2** in the same ratio.

Tinotuberide (6)———The butanol fraction was subjected to DCCC ($CHCl_3$/MeOH/H_2O, 35:65:40; upper layer as moving phase). A separated fraction containing the major constituent was chromatographed over a silicic acid column and eluted with a mixed solvent, $CHCl_3$/MeOH/H_2O (8:2:0.2), to give tinotuberide (**6**), coloress needles (from aqueous MeOH), mp 194—195°C $[\alpha]_D^{18}$ −15.6° (c=1.00, MeOH). *Anal.* Calcd for $C_{18}H_{26}O_{10}$: C, 53.72; H, 6.51. Found: C, 53.99; H, 6.4O. IR $\nu_{max}^{KBr}cm^{-1}$: 3600—3125 (OH), 1657 (C=C), 1590 (aromatic ring). UV λ_{max}^{MeOH} nm(ϵ): 221 (33700), 265 (16700). ^{1}H-NMR: Table II. ^{13}C-NMR: Table III.

Hydrogenaion of **6** (370 mg) over Pd–C in MeOH gave dihydrotinotuberide (**7**; 221 mg), colorless needles (from EtOH), mp 157.5—158.5°C, $[\alpha]_D^{24}$ −21.9° (c=1.86, MeOH). *Anal.* Calcd for $C_{18}H_{28}O_{10}$: C, 53.46; H, 6.98. Found: C, 53.30; H, 7.22. IR $\nu_{max}^{KBr}cm^{-1}$: 3550—3200 (OH), 1590 (aromatic ring). ^{1}H-NMR: Table II. ^{13}C-NMR: Table III.

Acetylatin of **6** with acetic anhydride in pyridine gave tinotuberide pentaacetate (**8**), colorless needles (from aqueous MeOH), mp 110.5—111.5°C, $[\alpha]_D^{25}$ −14.2° (c=2.02, $CHCl_3$). *Anal.* Calcd for $C_{28}H_{36}O_{15}$: C, 54.88, H, 5.92. Found: C, 55.29; H, 5.95. IR $\nu_{max}^{Nujol}cm^{-1}$: 1745, 1595. ^{1}H-NMR: Table II. ^{13}C-NMR: Table III. MS m/z: 521, 360, 331, 271, 252, 169, 127, 109, 85, 83, 58.

Enzymatic Hydrolysis of 6———Compound **6** (400 mg) was shaken with β-glucosidase (Sigma G-8625, from almond) in H_2O for 22 h at 37°C. The reaction mixture was subjected to DCCC ($CHCl_3$/MeOH/H_2O, 65:35:20; lower layer as moving phase) and separated to two fractions. The polar fraction was concentrated and chromatographed over activated charcoal (H_2O) to give D-glucose, white powder (from acetone), $[\alpha]_D^{25}$ +50.0° (c=2.0, H_2O). This was indistinguishable from commercial D-glucose on paper chromatography.

The less polar fraction was concentrated and chromatographed over silicic acid with hexane/EtOAc (1:1) to give 3-(4′-hydroxy-3′,5′-dimethoxyphenylmethoxy)-2-*trans*-propen-1-ol (**9**), pale yellow oil (85 mg). IR $\nu_{max}^{CHCl_3}cm^{-1}$: 3600—3500 (OH), 1655 (C=C), 1615 (aromatic ring). MS m/z: 222 (M−18$^+$), 183, 153.

Acetylatin of **9** (20 mg) with acetic anhydride in pyridine gave its diacetate (**10**; 5 mg), pale yellow oil. ^{1}H-NMR: Table II. ^{13}C-NMR: Table III.

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