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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, Tintotuberide

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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, tintotuberide (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; tintotuberide; 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, Bacon³⁾ reported the presence of a glycosidic bitter principle. Two alkaloids, named tinosporine and tinosporidine,⁴⁾ picoretin as its bitter principle,⁵⁾ waxes, hydrocarbons and a phytosterol⁶⁾ 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, tintotuberide. 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

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 $91^\circ C$, gave a diacetate (**3**), colorless needles, $C_{22}H_{23}O_6N$, mp $157^\circ C$, on acetylation, and tyramine (*p*-hydroxyphenethyl amine) as its hydrochloride, mp $240\text{--}242^\circ C$, on hydrolysis with hydrogen chloride in ethanol. Physical data derived from the infrared (IR) spectrum, ultraviolet (UV) spectrum, 1H -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 1H -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 elucidated 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	Multi- plicity	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 ₃ O	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	20.5	20.5		
				21.0	21.0	21.0		
CO	s			168.4	168.8	169.5		
				169.3	169.5	171.9		

a) Measured in DMSO- d_6 at 22.5 MHz.

b) Measured in CDCl₃ at 22.5 MHz.

c, d) Assignments may be reversed in each column.

TABLE II. ^1H -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)
$\text{CH}_3\text{O}-, \alpha$	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)
$\text{CH}_3\text{CO}-$		2.03s, 2.04s 2.10s (15H)		2.10s, 2.33s (6H)	

a) Measured in $\text{DMSO}-d_6$ 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 chromatography, and a new phenolic glucoside, named tinotuberide (6), colorless needles, $\text{C}_{18}\text{H}_{26}\text{O}_{10}$, mp 194—195°C, $[\alpha]_D^{18} - 15.6^\circ$ (MeOH), was obtained. Tinotuberide (6) gave a dihydro compound (7) on hydrogenation, and a pentaacetate (8), $\text{C}_{28}\text{H}_{36}\text{O}_{15}$, mp 110.5—111.5°C, $[\alpha]_D^{25} - 14.2^\circ$ (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, $[\alpha]_D^{25} + 50^\circ$, were obtained. In the ^1H -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 ^1H -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 ^1H -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).

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

Carbon	Multi- plicity	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 ₃ O-	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 ₃ CO-	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 CDCl₃ 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 Bangkok⁷⁾) were powdered and extracted with MeOH under reflux. After concentration *in vacuo*, the MeOH extract (1790 g) was dissolved in H₂O, and the solution was extracted successively with hexane, EtOAc and butanol. The EtOAc fraction was chromatographed over a silicic acid column and eluted with CHCl₃/MeOH/H₂O (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, H₂O/MeOH, 55:45).

***N-trans*-Feruloyl Tyramine (1)**—Colorless plates (from CHCl₃/MeOH), mp 91°C. *Anal.* Calcd for C₁₈H₁₉NO₄·CHCl₃: C, 52.73; H, 4.66; N, 3.24. Found: C, 52.96; H, 4.47; N, 3.40. IR $\nu_{\text{max}}^{\text{Nujol}}$ cm⁻¹: 3200, 1655, 1548. UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm(ϵ): 285 (11900), 293 (11900), 317 (13300). ¹H-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). ¹³C-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 C₂₂H₂₃NO₆: C, 66.49; H, 5.83; N, 3.52. Found: C, 66.43; H, 5.82; N, 3.43. IR $\nu_{\text{max}}^{\text{Nujol}}$ cm⁻¹: 1770, 1658, 1560. ¹H-NMR (CDCl₃) 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). ¹³C-NMR: Table I. MS *m/z*: 397 (M⁺), 355, 192, 177, 120. This product was identical with an authentic sample.⁸⁾

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 $\nu_{\text{max}}^{\text{Nujol}}$ cm^{-1} : 3275, 1755, 1640, 1545. $^1\text{H-NMR}$ (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). $^{13}\text{C-NMR}$: 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 $\nu_{\text{max}}^{\text{KBr}}$ 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 $\nu_{\text{max}}^{\text{film}}$ cm^{-1} : 3400–3100, 1645, 1585. $^1\text{H-NMR}$ ($\text{DMSO}-d_6$) 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). $^{13}\text{C-NMR}$: Table I. MS m/z : 313 (M^+), 193, 192, 177, 145, 120, 108, 107.

Acetylation of **2** with acetic anhydride in pyridine gave *N*-cis-feruloyl tyramine diacetate (**4**) as an oil. IR $\nu_{\text{max}}^{\text{film}}$ cm^{-1} : 1750, 1650, 1200. $^1\text{H-NMR}$ (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). $^{13}\text{C-NMR}$: 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 ($\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$, 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, $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$ (8:2:0.2), to give tinotuberide (**6**), colorless needles (from aqueous MeOH), mp 194–195°C [α_D^{18} -15.6° ($c=1.00$, MeOH)]. *Anal.* Calcd for $\text{C}_{18}\text{H}_{26}\text{O}_{10}$: C, 53.72; H, 6.51. Found: C, 53.99; H, 6.40. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3600–3125 (OH), 1657 (C=C), 1590 (aromatic ring). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm(ϵ): 221 (33700), 265 (16700). $^1\text{H-NMR}$: Table II. $^{13}\text{C-NMR}$: Table III.

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

Acetylation of **6** with acetic anhydride in pyridine gave tinotuberide pentaacetate (**8**), colorless needles (from aqueous MeOH), mp 110.5–111.5°C, [α_D^{25} -14.2° ($c=2.02$, CHCl_3)]. *Anal.* Calcd for $\text{C}_{28}\text{H}_{36}\text{O}_{15}$: C, 54.88, H, 5.92. Found: C, 55.29; H, 5.95. IR $\nu_{\text{max}}^{\text{Nujol}}$ cm^{-1} : 1745, 1595. $^1\text{H-NMR}$: Table II. $^{13}\text{C-NMR}$: 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 ($\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$, 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), [α_D^{25} $+50.0^\circ$ ($c=2.0$, 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 $\nu_{\text{max}}^{\text{CHCl}_3}$ cm^{-1} : 3600–3500 (OH), 1655 (C=C), 1615 (aromatic ring). MS m/z : 222 ($M-18^+$), 183, 153.

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

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