Studies on Nepalese Crude Drugs. XXVIII.[1] Chemical Constituents of Bhote Khair, the Underground Parts of *Eskemukerjea megacarpum* Hara[2]

Yukinori Miyaichi,*,[a] Norihisa Nunomura,[a] Yukio Kawata,[a] Haruhisa Kizu,[a] Tsuyoshi Tomimori,[a] Takashi Watanabe,[b] Akihito Takano,[c] and Kuber Jung Malla[d]

[a] *Department of Pharmacognosy, Pharmaceutical Sciences, Hokuriku University; Ho-3, Kanagawa-machi, Kanazawa, Ishikawa 920–1181, Japan:* [b] *Medicinal Plant Garden, Kitasato University School of Pharmaceutical Sciences; 1–15–1 Kitasato, Sagamihara, Kanagawa 228, Japan:* [c] *Medicinal Plant Garden, Showa College of Pharmaceutical Sciences; 3 Higashi-tamagawagakuen, Machida, Tokyo 194, Japan: and* [d] *Royal Botanical Garden, Department of Plant Resources; Godawari, Lalitpur, Nepal.* Received August 22, 2005; accepted October 14, 2005

From the underground parts of *Eskemukerjea megacarpum* Hara, two new stilbenes (14, 15) were isolated, together with a known coumarin, 5,7-dihydroxycoumarin (1), a tyramine derivative, *trans*-feruloyltyramine (2), two pyrogallol derivatives, gallic acid (3) and β-glucogallin (4), four flavonoids, trifolin (5), hyperin (6), myricetin 3-*O*-β-D-galactopyranoside (7), and myricitrin (8), five stilbenes, resveratorol (9), astringenin (10), piceid (11) astringin (12), and resveratorol 3-*O*-β-D-(6-*O*-galloyl)glucopyranoside (13), a flavan-3-ol, (−)-epigallocatechin 3-*O*-gallate (16), two proanthocyanidins, catechin-(4α→8)-epigallocatechin 3-*O*-gallate (17) and epicatechin 3-*O*-gallate-(4β→8)-epigallocatechin 3-*O*-gallate (18), and an anthocyanin, idaein (19). Compounds 14 and 15 were identified as (*E*)-3,5,3′,4′-tetrahydroxystilbene 3-*O*-β-D-(6-*O*-galloyl)glucopyranoside and (*E*)-3,5,4′-trihydroxystilbene 3-*O*-β-D-(6-*O*-galloyl)glucopyranoside, respectively, based on spectral and chemical data.

Key words *Eskemukerjea megacarpum*; stilbene; coumarin; favonoid; proanthocyanidin; anthocyanin

Eskemukerjea megacarpum Hara is a perennial herb of the family Polygonaceae, growing at 2000—3000 m in the area of western and central Nepal.[3,4] In Nepal, this plant is called Bhote Khair and used for an excess bleeding control during menstruation as a traditional medicine.

As regards the constituents of this plant, no work has been reported to the best of our knowledge. In our studies on Nepalese crude drugs, we isolated two new stilbenes (**14**, **15**) together with a known coumarin (**1**), a tyramine derivative (**2**), two pyrogallol derivatives (**3**, **4**), four flavonoids (**5**—**8**), five stilbenes (**9**—**13**), a flavan-3-ol (**16**), and two proanthocyanidins (**17**, **18**) from an extract of MeOH and an anthocyanin (**19**) from that of H_2O–HCOOH (20 : 1) of the underground parts in this plant as described in Experimental. This paper deals with the identification of their structures.

The structures of the known compounds were identified as 5,7-dihydroxycoumarin (**1**),[5] *trans*-feruloyltyramine (**2**),[6] gallic acid (**3**),[7] β-glucogallin (**4**),[7] trifolin (**5**),[8] hyperin (**6**),[9,10] myricetin 3-*O*-β-D-galactopyranoside (**7**),[11,12] myricitrin (**8**),[11] resveratorol (**9**),[13] astringenin (**10**),[14] piceid (**11**),[15] astringin (**12**),[16] resveratorol 3-*O*-β-D-(6-*O*-galloyl)glucopyranoside (**13**),[17,18] (−)-epigallocatechin 3-*O*-gallate (**16**),[19] catechin-(4α→8)-epigallocatechin 3-*O*-gallate (**17**),[19] epicatechin 3-*O*-gallate-(4β→8)-epigallocatechin 3-*O*-gallate (**18**),[19] and idaein (**19**)[20] by direct comparison with authentic samples or of the respective spectral and chemical data with those described in the literature. Compounds **14** and **15** gave the IR absorption bands assignable to hydroxyl, conjugated carbonyl groups, and aromatic rings in IR spectra. The UV spectra of **14** and **15** were characteristic of stilbene series.[21]

Compounnd **14** was obtained as colorless needles, $C_{27}H_{26}O_{13}$. The ^{1}H- and ^{13}C-NMR signal patterns revealed the presence of (*E*)-3,5,3′,4′-tetrahydroxystilbene, a galloyl group, and glucopyranose moiety in **14**. Enzymatic hydrolysis of **14** with tannase afforded gallic acid (**3**) and astringin (**12**). On acid hydrolysis, **14** gave **3**, astringenin (**10**), and D-glucose.

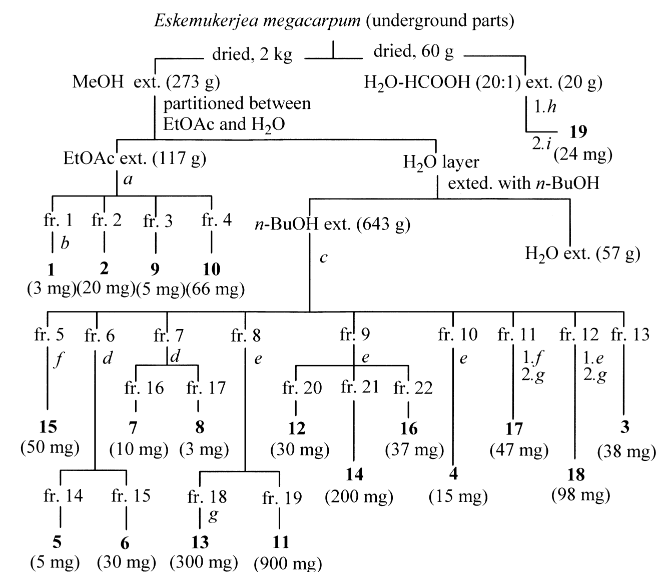
A detailed comparison of the ^{1}H- and ^{13}C-NMR spectra of **14** with those of **12** revealed that the linkage site of the galloyl group in **14** was at the C-6 position of the glucopyranose moiety of **12**: acylation shifts were observed at the H_2-6″ (+0.66, +0.86 ppm), C-6″ (+2.1 ppm), and C-5″ (−3.5 ppm). An anomeric proton signal of **14** was observed at δ 4.92 (1H, d, J=7.6 Hz), and the J value indicated that the glycosidic linkage was of the β-configuration. From these results, the structure of **14** was concluded to be (*E*)-3,5,3′,4′-tetrahydroxystilbene 3-*O*-β-D-(6-*O*-galloyl)glucopyranoside.

Compounnd **15** was obtained as colorless needles, $C_{29}H_{30}O_{12}$. In a comparison of the ^{1}H- and ^{13}C-NMR data for **15** with those of resveratorol 3-*O*-β-D-(6-*O*-galloyl)glucopyranoside (**13**), **15** showed the presence of (*E*)-3,5,4′-trihydroxystilbene glucoside with a syringyl group. Compound **15** gave syringic acid and piceid (**11**) on alkaline hydrolysis and afforded syringic acid, resveratorol (**9**), and D-glucose on acid hydrolysis. Thus **15** was a syringate of **11**. In the ^{1}H- and ^{13}C-NMR spectra of **15**, acylation shifts were observed at H_2-6″ (+0.95, +0.70 ppm), C-6″ (+3.4 ppm), and C-5″ (−3.4 ppm) compared with those of **11**. A syringyloxy group was deduced to be linking to the C-6 position of the glucose of **11**. From these results, the structure of **15** was concluded to be (*E*)-3,5,4′-trihydroxystilbene 3-*O*-β-D-(6-*O*-galloyl)glucopyranoside.

Experimental

General Procedures All melting points were determined on a Yanagimoto micromelting point apparatus and a uncorrected. NMR spectra were recorded in DMSO-d_6 on a JEOL GSX-400 spectrometer (^{1}H-NMR at 400 MHz and ^{13}C-NMR at 100 MHz), using TMS as an internal standard. MS were recorded on a JEOL JMS-SX-102A mass spectrometer, and UV spectra on a Shimadzu dual-wavelength/double-beam recording spectrophotometer. Samples for IR spectra were prepared as a KBr disk, and the spectra were recorded on a Hitachi 270-30 infrared spectrophotometer. Optical rota-

∗ To whom correspondence should be addressed. e-mail: y-miyaichi@hokuriku-u.ac.jp © 2006 Pharmaceutical Society of Japan



Chromatog. condition
a: SiO_2 column [$CHCl_3$-MeOH-H_2O] (1:0:0→6:1:0.1)
b: SiO_2 column [benxene-EtOAc](1:0→1:1)
c: SiO_2 column [$CHCl_3$-MeOH-H_2O] (10:1:0.1→2:1:0.1)
d: ODS column [H_2O-MeOH](1:0→10:1)
e: SiO_2 column [EtOAc-acetone-HCOOH-H_2O](30:1:0.5:0.5→2:1:0.5:0.5)
f: SiO_2 column [EtOAc-acetone-HCOOH-H_2O](40:1:0.5:0.5→30:1:0.5:0.5)
g: ODS column [H_2O-MeOH](10:1→1:1.5)
h: ODS column [H_2O-THF-10%HCl](10:0:0.5→5:1:0.5)
i: ODS column [H_2O-MeOH-HCOOH](10:0:0.5→10:1:0.5)]

Chart 1

tion was measured with a JASCO DIP-370 digital polarimeter. For column chromatography, silica gel (Wako-gel C-300) and ODS (Cosmosil 140 C_{18}-OPN) were used. HPLC analysis of sugar was carried out under the following conditions: column, YMC-Pack Polyamine-II (250 mm×4.6 mm i.d.); solvent, $CH_3CN : H_2O : H_3PO_4 = 86 : 14 : 0.05$; detector, Shimadzu RID-2A index detector and JASCO OR-990 optical rotation detector; and temperature, 50 °C, which revealed the presence of D-glucose (t_R 16.9 min, $\alpha+$).

The materials were collected in central Nepal and purchased in Kathmandu (commercial name: Bademul), Nepal. The TLC pattern of the latter crude drugs showed good agreement with that of the underground parts of the former plants. A voucher specimen is deposited at the Herbarium of the Faculty of Pharmaceutical Sciences, Hokuriku University, Kanazawa, Japan. An aqueous suspension of a hot MeOH extract of both materials was extracted successively with ethyl acetate and *n*-butanol. The ethyl acetate and *n*-butanol extract were chromatographed, respectively, to give **1**—**18**, as shown in Chart 1. Moreover, to investigate the red-colored substance, both materials were extracted with H_2O–HCOOH (20 : 1) as solvent and separated with ODS to give **19**.

Compound 14 Colorless needles, mp 173—174 °C, $[\alpha]_D^{27}$ −84.6° (c=0.14, MeOH). IR (KBr) cm^{-1}: 3400, 1691, 1612. FAB-MS *m/z*: 559 $[(M+H)^+]$, 581 $[(M+Na)^+]$, HR-FAB-MS *m/z*: 559.1459 (Calcd for $C_{27}H_{27}O_{13}$, 559.1452); *m/z*: 581.1266 (Calcd for $C_{27}H_{26}O_{13}Na$, 581.1272). UV λ_{max} nm (log ε): 293 (4.59), 303 (4.59), 326 (4.60). ^{13}C-NMR: Table 1. ^1H-NMR: Table 2.

Enzymatic Hydrolysis of 14 To a solution of **14** (5 mg) in H_2O (5 ml) tannase (1 mg, *Aspergillus oryzae*, Wako) was added and the mixture was allowed to stand at 37 °C for 8 h. The reaction mixture was extracted with *n*-BuOH. The *n*-BuOH layer gave two spots on TLC (developed with $CHCl_3$–MeOH–H_2O–HCOOH=6 : 1 : 0.3 : 0.1), which were identified as gallic acid (**3**, *Rf*=0.30) and astringin (**12**, *Rf*=0.21).

Acid Hydrolysis of 14 Compound **14** (3 mg) was dissolved in 2 N H_2SO_4 (3 ml) and heated at 90 °C for 2 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 and extracted with *n*-BuOH. The *n*-BuOH layer was processed in the same manner as for enzymatic hydrolysis to give two spots, which were identified as gallic acid (**3**, *Rf*=0.30) and astringenin (**10**, *Rf*=0.61). The aqueous layer showed the peak of D-glucose on HPLC analysis.

Table 1. ^{13}C-Chemical Shifts of Compounds **11**—**15**, δ (ppm) in DMSO-d_6

C-No.	**11**	**12**	**13**	**14**	**15**
1	139.2	139.3	139.2	139.4	139.4
2	104.7	104.8	105.1	105.2	104.8
3	158.9	158.9	158.7	158.7	158.7
4	102.7	102.7	102.7	102.7	102.6
5	158.3	158.3	158.4	158.4	158.4
6	107.1	107.1	106.8	106.8	106.8
α	128.5	128.9	128.6	128.9	128.5
α'	125.2	125.1	125.2	125.1	125.1
1′	128.0	128.5	127.9	128.5	127.8
2′	127.9	113.3	127.9	113.5	127.8
3′	115.5	145.4	115.5	145.4	115.5
4′	157.3	145.6	157.3	145.6	157.3
5′	115.5	115.7	115.5	115.7	115.5
6′	127.8	118.7	127.9	118.6	127.9
Glc-1	100.7	100.7	100.6	100.6	100.2
2	73.3	73.3	73.3	73.3	73.1
3	76.7	76.7	76.3	76.3	76.2
4	69.8	69.8	69.2	69.1	70.2
5	77.1	77.1	73.7	73.6	73.8
6	60.7	60.7	62.9	62.8	64.1
Acyl-1			119.3	119.4	119.2
2, 6			108.6	108.6	106.8
3, 5			145.5	145.5	147.5
4			138.5	138.5	140.8
C=O			165.8	165.8	165.5
3, 5-OMe					55.9

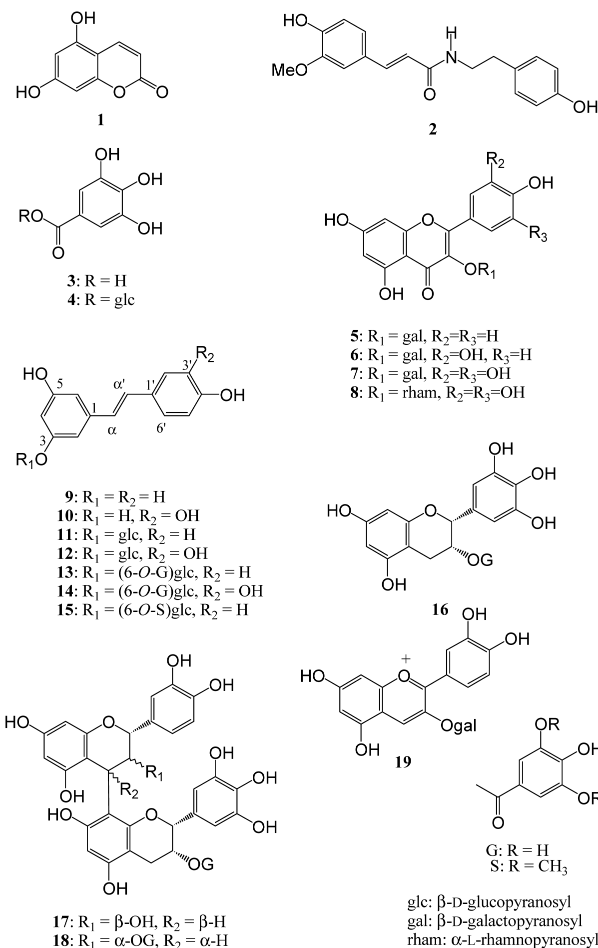


Chart 2

Table 2. ^{1}H-Chemical Shifts of Compounds **11**—**15** δ (ppm) in DMSO-d_6 (J/Hz in Parentheses)

H-No.	**11**	**12**	**13**	**14**	**15**
2	6.73 br s	6.73 br s	6.63 br s	6.64 br s	6.64 br s
4	6.34 dd (2.0, 2.0)	6.33 dd (2.0, 2.0)	6.35 dd (2.0, 2.0)	6.35 dd (2.0, 2.0)	6.35 dd (2.0, 2.0)
6	6.56 br s	6.56 br s	6.58 br s	6.58 br s	6.57 br s
α	6.87 d (16.4)	6.77 d (16.4)	6.82 d (16.8)	6.75 d (16.4)	6.77 d (16.4)
α'	7.03 d (16.4)	6.96 d (16.4)	6.98 d (16.8)	6.92 d (16.4)	6.96 d (16.4)
2′	7.40 d (8.8)	6.97 d (2.0)	7.36 d (8.8)	6.97 d (2.0)	7.30 d (8.8)
3′	6.76 d (8.8)		6.75 d (8.8)		6.74 d (8.8)
5′	6.76 d (8.8)	6.72 d (8.4)	6.75 d (8.8)	6.72 d (8.4)	6.74 d (8.8)
6′	7.40 d (8.8)	6.84 dd (8.4, 2.0)	7.36 d (8.8)	6.82 dd (8.4, 2.0)	7.30 d (8.8)
Glc-1	4.80 d (7.6)	4.80 d (7.6)	4.90 d (7.6)	4.92 d (7.6)	4.95 d (8.0)
2	3.16—3.36 m	3.16—3.36 m	3.27 dd (8.2, 7.6)	3.28 m	3.23—3.37 m
3	3.16—3.36 m	3.16—3.36 m	3.34 dd (9.0, 8.2)	3.35 m	3.23—3.37 m
4	3.16—3.36 m	3.16—3.36 m	3.41 dd (9.2, 9.0)	3.43 m	3.23—3.37 m
5	3.16—3.36 m	3.16—3.36 m	3.68 ddd (9.2, 3.5, 2.0)	3.68 m	3.23—3.37 m
6	3.73 dd (12.0, 4.0)	3.73 br d (12.0)	4.40 dd (12.0, 2.0)	4.39 dd (12.0, 2.0)	4.68 dd (12.0, 2.0)
	3.49 dd (12.0, 6.0)	3.49 m	4.33 dd (12.0, 3.5)	4.35 dd (12.0, 3.0)	4.19 dd (12.0, 3.2)
Acyl-2, 6			6.97 s	6.97 s	7.19 s
3, 5-OMe					3.73 s

Compound 15 Colorless needles, mp 168—169 °C, $[\alpha]_D^{27}$ −60.3° (c=0.12, MeOH). IR (KBr) cm^{-1}: 3408, 1697, 1606, 1595. FAB-MS m/z: 571 [(M+H)$^+$], 593 [(M+Na)$^+$], HR-FAB-MS m/z: 571.1808 (Calcd for $C_{29}H_{31}O_{12}$, 571.1808); m/z: 593.1647 (Calcd for $C_{29}H_{30}O_{12}Na$, 593.1636). UV λ_{max} nm (log ε): 296 (4.48), 303 (4.47), 320 (4.42). ^{13}C-NMR: Table 1. ^{1}H-NMR: Table 2.

Alkaline Hydrolysis of 15 Compound **15** (2 mg) was dissolved in 0.5 N KOH (5 ml) and allowed to stand at room temperature for 5 h. The reaction mixture was neutralized with HCOOH and extracted with n-BuOH. The n-BuOH layer showed two spots on TLC (developed with $CHCl_3$–MeOH–H_2O–HCOOH=6 : 1 : 0.3 : 0.1), which were identified as syringic acid (Rf=0.58) and piceid (**11**, Rf=0.23).

Acid Hydrolysis of 15 Compound **15** (3 mg) was processed in the same manner as **14** to give two spots, which were identified as syringic acid (Rf=0.58) and resveratrol (**9**, Rf=0.67). The aqueous layer showed the peak of D-glucose on HPLC analysis.

Identification of 1—13 and 16—19 Compounds **1**—**13** and **16**—**19** were identified as 5,7-dihydroxycoumarin (**1**),[5] *trans*-feruloyltyramine (**2**),[6] gallic acid (**3**),[7] β-glucogallin (**4**),[7] trifolin (**5**),[8] hyperin (**6**),[9,10] myricetin 3-*O*-β-D-galactopyranoside (**7**),[11,12] myricitrin (**8**),[11] resveratorol (**9**),[13] astringenin (**10**),[14] piceid (**11**),[15] astringin (**12**),[16] resveratorol 3-*O*-β-D-(6-*O*-galloyl)glucopyranoside (**13**),[17,18] (−)-epigallocatechin 3-*O*-gallate (**16**),[19] catechin (4α→8)-epigallocatechin 3-*O*-gallate (**17**),[19] epicatechin 3-*O*-gallate-(4β→8)-epigallocatechin 3-*O*-gallate (**18**),[19] and idaein (**19**),[20] respectively, by direct comparison (UV, IR, and ^{1}H- and ^{13}C-NMR spectra) with authentic samples (**3**, **5**, **6**, **8**, **16**) or of respective spectral and chemical data with those described in the literature (**1**, **2**, **4**, **7**, **9**—**13**, **17**—**19**).

Acknowledgments We are grateful to the staff of the analytical center of Hokuriku University for MS measurements.

References and Notes

1) Part XXVII: Kizu H., Tomimori T., *Natu. Med.*, **57**, 118 (2003).
2) Abstract of Papers, the 46th Annual Meeting of the Japanese Society of Pharmacognosy, Osaka, September 1999, p. 116.
3) Shrestha T. B., Joshin R. M., “Endemic and Endangered Plants of Nepal,” WWF Nepal Program, Kathmandu, 1996, p. 135.
4) Hara H., *Enum. Fl. Pl.* (Nepal), **3**, 173 (1982).
5) Bilbao J. L. G., Rodriguez B., *An. Quim*, **74**, 1570—1572 (1978).
6) Chen C. Y., Chang F. R., Wu Y. C., *J. Chin. Chem. Soc.*, **44**, 313—319 (1997).
7) Fischer E., Bergmann M., *Ber.*, **51**, 1760—1804 (1918).
8) Lin C. N., Arisawa M., Shimizu M., Morita N., *Phytochemistry*, **21**, 1466—1469 (1981).
9) Marco J. A., Barbera O., Sanz J. F., Parareda J. S., *Phytochemistry*, **24**, 2471—2472 (1985).
10) Markham K. R., Ternai B., Stanley R., Geiger H., Mabry T. J., *Tetrahedron*, **34**, 1389—1397 (1978).
11) Agrawal P. K., “Carbon-13 NMR of Flavonoids,” Elsevier Science Publishers B. V., Amsterdam, 1989, pp. 128—130.
12) Osima Y., Nakabayashi T., *J. Agric. Chem. Soc. Jpn.*, **27**, 754—756 (1953).
13) Ito T., *Yakugaku Zasshi*, **81**, 236—238 (1961).
14) Cunningham J., Haslam E., Haworth R. D., *J. Chem. Soc.*, **1958**, 2875—2883 (1958).
15) Kariyone T., Takahashi M., Ito T., Masutani K., *Yakugaku Zasshi*, **78**, 935—937 (1958).
16) Manners G. D., Swant E. P., *Phytochemistry*, **10**, 607—610 (1971).
17) Okasaka M., Takaishi Y., Kogure K., Fukuzawa K., Shibata H., Higuti T., Honda G., Ito M., Kodzhimatov K. O., Ashurmefov O., *J. Nat. Prod.*, **67**, 1044—1046 (2004).
18) Hegde V. R., Pu H., Patel M., Black T., Soriano A., Zhao W., Gullo V. D., Chan T. M., *Bioorg. Med. Chem. Lett.*, **14**, 2275—2277 (2004).
19) Hashimoto F., Nonaka G., Nishioka I., *Chem. Pharm. Bull.*, **37**, 3255—3263 (1989).
20) Loose R., *Phytochemistry*, **9**, 875—879 (1970).
21) Braude E. A., *J. Chem. Soc.*, **1949**, 1902—1909 (1949).