Isolation of Two New Coumarin Glycosides from *Notopterygium forbesii* and Evaluation of a Chinese Crude Drug, Qiang-Huo, the Underground Parts of *N. incisum* and *N. forbesii*, by High-Performance Liquid Chromatography

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From the ether extract of the underground part of *Notopterygium forbesii*, two new coumarin glycosides, bergaptol-*O*-β-D-glucopyranoside and 6′-*O*-*trans*-feruloylnodakenin, were isolated along with known compounds including seven furanocoumarins, two dihydrofuranocoumarins, a sterol glucoside and two phenolic compounds. Analysis of their contents by high-performance liquid chromatography (HPLC) revealed that the underground part of *N. forbesii* contained large amounts of *p*-hydroxyphenethyl anisate (0.7%), bergaptol glucoside (0.2%), nodakenin (2%) and 6′-*O*-*trans*-feruloylnodakenin (0.7%) and a lesser amount of notopterol (0.08%), while that of *N. incisum* contained a large amount of notopterol (1.2%) and less amounts of the others. The characteristic difference in chemical composition between the two species enabled us to identify the respective botanical sources of a Chinese crude drug, Qiang-huo derived from *N. incisum* and *N. forbesii* by HPLC.

Keywords bergaptol glucoside; crude-drug evaluation; 6′-*O*-feruloylnodakenin; furanocoumarin; HPLC; *Notopterygium forbesii*; *Notopterygium incisum*

Qiang-huo (羌活; Kyokatsu in Japanese) is a well-known Chinese crude drug widely used as an antirheumatic and pain-relieving agent for the treatment of colds and rheumatism. The drug produced in China is made of the underground portion of two species of *Notopterygium* (Umbelliferae), *N. incisum* TING *ex* CHANG and *N. forbesii* DE BOISS.

As regards the chemical constituents of Qiang-huo, sugars, amino acids, organic acids,[1] mono- and sesquiterpenes,[2] a polyacetylene compound,[3,4] coumarins[3,4] and phenolic compounds[3,4] were reported to be present in the underground part of *N. incisum*. Few studies, however, have been performed on the constituents of *N. forbesii*.

We describe here the isolation and characterization of two new compounds from the underground part of *N. forbesii*, and the evaluation of various Qiang-huo products available in Chinese markets by high-performance liquid chromatography (HPLC).

Results and Discussion

Constituents of the Underground Part of *N. forbesii* Repeated column chromatography on silica gel followed by preparative thin-layer chromatography (TLC) led to the isolation of two new compounds and twelve known compounds, bergamottin (**1**), isoimperatorin (**2**), cnidilin (**3**), *p*-hydroxyphenethyl anisate (**4**), notopterol (**5**), bergapten (**6**), *trans*-ferulic acid (**7**), demethylfuropinnarin (**8**), bergaptol (**9**), nodakenetin (**10**), β-sitosterol glucoside (**11**) and nodakenin (**12**). Characterization of the new compounds (**13** and **14**) was performed by chemical and

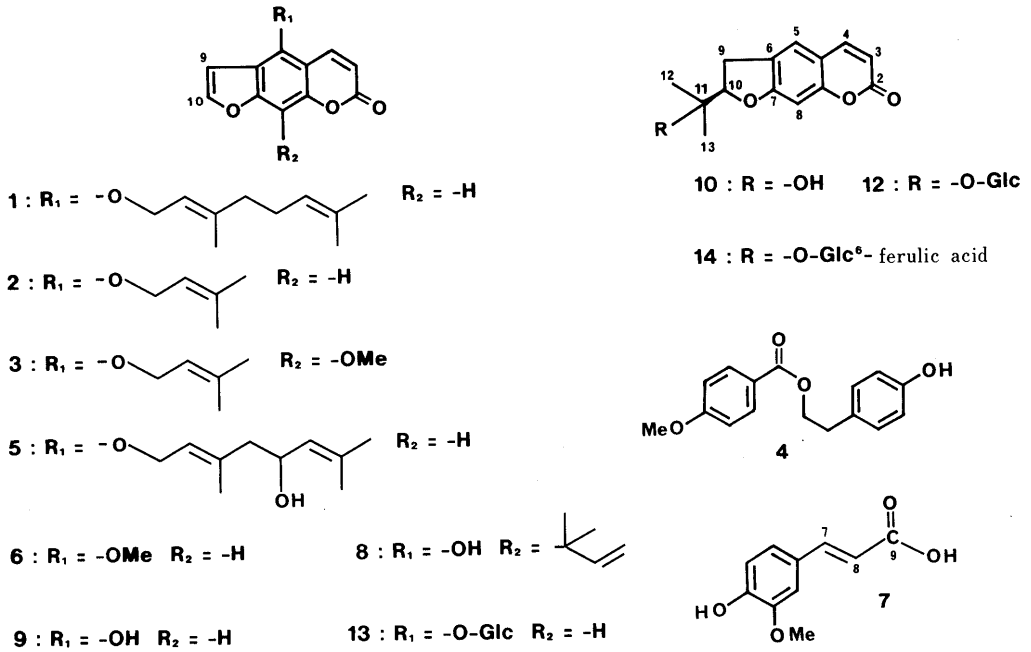

Chart 1. Structures of Coumarins and Phenolic Compounds Isolated from the Underground Part of *N. forbesii*

spectroscopic means as follows.

Compound **13** was obtained as colorless needles, mp 254—256 °C. Its fast-atom-bombardment mass spectrum (FAB-MS) showed the quasi molecular ion peak at *m/z* 365, corresponding to $C_{17}H_{16}O_9+H$. The proton and carbon-13 nuclear magnetic resonance spectra (^{1}H- and ^{13}C-NMR) (Table I) showed the presence of a furanocoumarin ring and a glucopyranosyl moiety. The latter was also supported by the presence of a prominent fragmentation ion at *m/z* 185, due to the removal of a monosaccharide unit in the FAB-MS. On acid or enzymic hydrolysis, **13** gave bergaptol (**9**) and glucose, which were identified by direct comparison with authentic samples. On the basis of the coupling constant value ($J=7.3$ Hz) of the anomeric proton signal (δ 5.67, d) in **13**, the compound was concluded to be bergaptol-*O*-β-D-glucopyranoside.

Compound **14** was obtained as colorless needles, mp 138—140 °C. The compound gave a tetraacetate (**14a**) on acetylation. The ^{1}H- and ^{13}C-NMR spectra of **14** (Table I) showed the presence of a *trans*-feruloyl group, a glucopyranosyl moiety and a tetrahydrofuranocoumarin skeleton (nodakenetin or marmesin type). The compound gave *trans*-ferulic acid (**7**), glucose and nodakenetin (**10**) on acid hydrolysis, while it gave nodakenin (**12**) and methyl *trans*-ferulate on methanolysis. In comparison of the ^{13}C-NMR spectra of **12** and **14** (Table I), the signal at δ 63.7 (C-6′ of glucose) in **14** was appreciably shifted downfield by 2.5 units, indicating that the feruloyl group is attached to 6′-OH of the sugar. The structure of **14** was consequently established as 6′-*O*-*trans*-feruloylnodakenin.

TABLE I. ^{13}C-NMR Spectral Data for Compounds **12**—**14** and **14a**

Carbon	13	12	14	14a
Coumarin moiety				
2	160.0 (s)	160.4 (s)	160.4 (s)	161.2 (s)
3	112.7 (d)	112.2 (d)	112.3 (d)	111.9 (d)
4	140.0 (d)	144.6 (d)	144.5 (d)	143.6 (d)
4a	107.5 (s)	112.2 (s)	111.2 (s)	112.6 (s)
5	151.7 (s)	123.8 (d)	124.0 (d)	123.1 (d)
6	115.2 (s)	125.4 (s)	125.3 (s)	124.7 (s)
7	157.1 (s)	163.0 (s)	162.9 (s)	163.0 (s)
8	94.9 (d)	96.8 (d)	96.8 (d)	97.5 (d)[a]
8a	147.1 (s)	155.0 (s)	154.9 ((s)	155.4 (s)
9	105.4 (d)	29.1 (t)	29.2 (t)	29.5 (t)
10	146.4 (d)	89.7 (d)	89.6 (d)	89.7 (d)
11		77.0 (s)	77.2 (s)	78.5 (s)
12		20.3 (q)	20.9 (q)	21.2 (q)
13		23.2 (q)	23.0 (q)	23.3 (q)
Sugar moiety				
1′	103.9 (d)	97.2 (d)	97.1 (d)	95.4 (d)[a]
2′	73.8 (d)	73.5 (d)	73.4 (d)	71.2 (d)[b]
3′	76.2 (d)	76.6 (d)	76.6 (d)	72.8 (d)
4′	69.8 (d)	70.3 (d)	70.5 (d)	68.9 (d)
5′	77.4 (d)	76.9 (d)	77.2 (d)	71.6 (d)[b]
6′	60.8 (t)	61.2 (t)	63.7 (t)	62.4 (t)
Feruloyl moiety				
1″			125.2 (s)	123.3 (s)
2″			111.0 (d)	111.2 (d)
3″			149.3 (s)	151.3 (s)
4″			145.0 (s)	144.7 (s)
5″			114.1 (d)	132.8 (d)
6″			125.2 (d)	121.1 (d)
7″			147.8 (d)	141.6 (d)
8″			115.3 (d)	117.2 (d)
9″			166.4 (s)	165.9 (s)
$\underline{C}H_3O$–			55.6 (q)	55.8 (q)
Acetyl moiety				
CH_3–$\underline{C}O$–				170.0 (s)
				169.4 (s)
				168.9 (s)
				168.5 (s)
				20.3 (q)
$\underline{C}H_3$–CO–				20.5 (q)

Compounds **12**—**14** were measured in dimethyl sulfoxide-d_6 at 22.5 MHz and compound **14a** in $CDCl_3$. *a*, *b*) Assignments may be exchangeable.

Quantitative Analysis of Coumarins and Phenolic Compounds by HPLC For the purpose of evaluating the crude drugs derived from *Notopterygium* species, we

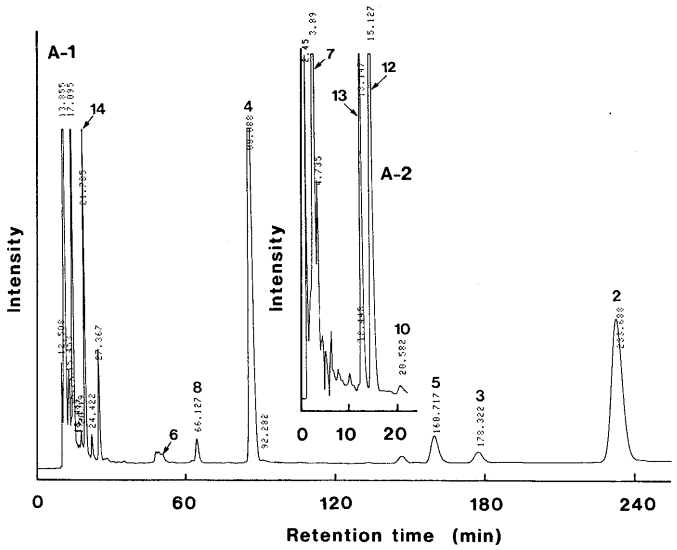

Fig. 1. An HPLC Elution Profile of a MeOH Extract of the Underground Part of *N. forbesii*

The MeOH extract was analyzed by HPLC (LichroCAT ODS column) with MeCN–MeOH–H_2O (34 : 6 : 60) (A-1) and MeCN–MeOH–H_2O (21 : 6 : 73) (A-2) as mobile phases. The elution profiles were monitored at 254 nm. **2**, isoimperatorin; **3**, cnidilin; **4**, *p*-hydroxyphenethyl anisate; **5**, notopterol; **6**, bergapten; **7**, ferulic acid; **8**, demethylfuropinnarin; **10**, nodakenetin; **12**, nodakenin; **13**, bergaptol glucoside; **14**, 6′-*O*-feruloylnodakenin.

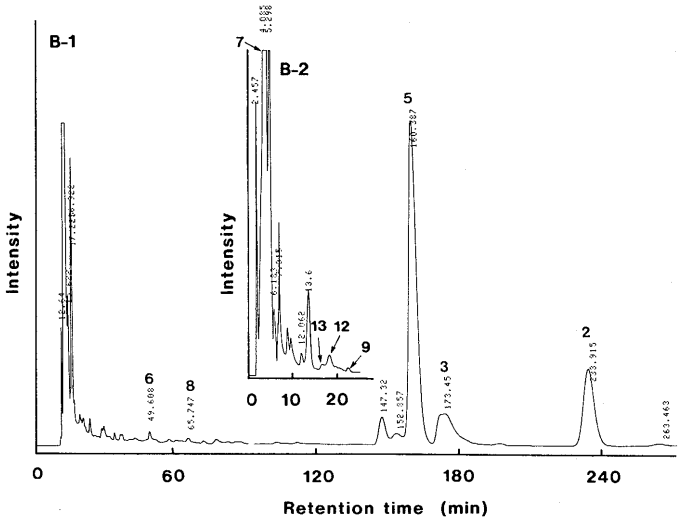

Fig. 2. An HPLC Elution Profile of a MeOH Extract of the Underground Part of *N. incisum*

The MeOH extract was analyzed under the same conditions as described in the legend to Fig. 1. The elution profile of B-1 was obtained with MeCN–MeOH–H_2O (34 : 6 : 60) as a mobile phase and that of B-2 with MeCN–MeOH–H_2O (21 : 6 : 73). **2**, isoimperatorin; **3**, cnidilin; **5**, notopterol; **6**, bergapten; **7**, ferulic acid; **8**, demethylfuropinnarin; **9**, bergaptol; **12**, nodakenin; **13**, bergaptol glucoside; **14**, 6′-*O*-feruloylnodakenin.

determined the quantity of coumarins and phenolic compounds in the commercially available drugs and the underground parts of *N. forbesii* and *N. incisum* by means of HPLC with an octadecyl silica (ODS) column. Figures 1 and 2 show typical elution profiles of the underground parts of *N. forbesii* and *N. incisum*, respectively. Each peak was identified by comparing the retention time with that of an authentic sample. The quantity of each compound was calculated from the respective standard curves. The standard curves showed good linearity between the peak-area and concentration of the compound. Their regression equations are shown in Table II. The accuracy of each equation was confirmed by recovery experiments (see Table III). The percentage recovery was 88.4—112.5% with correlation of variation (C.V.) in a range of 0.8—3.5% except for one case (C.V. = 8.1% for **8**). Both extracts of the underground parts of *N. incisum* and *N. forbesii* (samples I and VII, respectively) contained many common constituents though their overall contents were quite different from each other.

Next, we analyzed various extracts of commercially available Qiang-huo (samples II—VI and samples VIII—IX). The HPLC elution pattern of samples II—V and their contents of coumarins and phenolic compounds were quite similar to those of *N. incisum* (sample I), while the patterns of samples VIII and IX were similar to that of *N. forbesii*. Sample VI, which is a poor grade of Qiang-huo and may be the undergound part of *N. incisum*, contained less amounts of these constituents. These drugs obtained in Chinese markets were classified into two groups with respect to their content of coumarins and phenolic compounds as shown in Fig. 3: group A (samples II—VI) contained a large amount of notopterol (**5**) (*ca.* 0.7—1.2%), while group B contained large amounts of *p*-hydroxyphenethyl anisate (**4**) (*ca.* 1%), nodakenin (**12**) (2—3%), bergaptol glucoside (**13**) (*ca.* 0.3%), and 6′-*O*-*trans*-feruloyl nodakenin (**14**) (*ca.* 1%). There was no appreciable difference in the contents of isoimperatorin (**2**), *trans*-ferulic acid (**7**), bergaptol (**9**) and nodakenetin (**10**). The characteristic profiles of the chemical

TABLE II. Regression Equations and Their Correlation Coefficients for Quantitative Analysis of Coumarins and Phenolic Compounds

Compound	Equation of regression line (Y)	Correlation coefficient (r)
Isoimperatorin (**2**)	$469586X+168586$	0.987
Cnidilin (**3**)	$309617X+10150$	0.999
p-Hydroxyphenylethyl anisate (**4**)	$354854X+19106$	0.999
Notopterol (**5**)	$364202X+4402$	0.999
Bergapten (**6**)	$550264X+414$	0.999
Ferulic acid (**7**)	$228478X+10425$	0.999
Demethylfuropinnarin (**8**)	$381189X+5904$	0.951
Bergaptol (**9**)	$36665X+572$	0.999
Nodakenetin (**10**)	$142220X+1371$	0.999
Nodakenin (**12**)	$13399X+6293$	0.989
Bergaptol glucoside (**13**)	$519264X+15038$	0.999
6′-*O*-Feruloylnodakenin (**14**)	$107681X+3488$	0.999

X and Y represent the peak-area ($\mu V \cdot s$) and the concentration of the compound ($\mu g/ml$), respectively.

TABLE III. Accuracy of the Analysis of Coumarins and Phenolic Compounds by HPLC

Compound	Added amount (μg)	Found (μg)	Recovery (%)	C.V. (%)
Isoimperatorin (**2**)	0.034	0.032	94.1	3.5
Cnidilin (**3**)	0.330	0.310	93.9	2.6
p-Hydroxyphenetyl anisate (**4**)	0.430	0.412	95.8	0.9
Notopterol (**5**)	0.071	0.069	97.2	1.8
Bergapten (**6**)	0.242	0.214	88.4	1.7
Ferulic acid (**7**)	0.032	0.036	112.5	1.8
Demethylfuropinnarin (**8**)	0.113	0.120	106.2	8.1
Bergaptol (**9**)	0.252	0.246	97.6	1.7
Nodakenetin (**10**)	0.212	0.204	96.2	2.5
Nodakenin (**12**)	0.623	0.612	98.2	1.4
Bergaptol glucoside (**13**)	0.051	0.052	101.9	0.8
6′-*O*-Feruloylnodakenin (**14**)	0.294	0.280	95.2	1.3

TABLE IV. Contents of Coumarins and Phenolic Compounds in the Underground Parts of *N. incisum* and *N. forbesii* and in the Crude Drug Qiang-huo Available in Chinese Markets

Compound	Contents (mg/100 g of crude drug)								
	Sample (group A)						Sample (group B)		
	I	II	III	IV	V	VI	VII	VIII	IX
Isoimperatorin (**2**)	380.4	278.8	211.2	368.9	815.0	54.3	620.2	1010.0	277.4
Cnidilin (**3**)	337.0	330.5	173.5	96.0	230.5	Trace	24.0	108.5	47.5
p-Hydroxyphenethyl anisate (**4**)	5.1	13.1	2.2	6.4	6.8	1.9	703.5	991.5	1056.0
Notopterol (**5**)	1197.0	1117.0	696.0	661.0	885.5	83.0	83.5	131.5	6.5
Bergapten (**6**)	9.0	6.0	4.0	1.2	7.5	Trace	8.0	17.5	14.5
Ferulic acid (**7**)	890.6	874.6	1031.1	963.4	602.6	620.7	825.9	820.0	690.5
Demethylfuropinnarin (**8**)	11.5	12.5	11.0	13.0	14.0	Trace	38.0	15.0	14.5
Bergaptol (**9**)	88.0	28.5	69.0	53.0	58.5	29.0	26.5	57.5	36.0
Nodakenetin (**10**)	40.5	20.0	17.0	16.0	22.5	10.0	53.5	15.0	53.5
Nodakenin (**12**)	Trace	111.9	78.7	89.8	34.6	49.8	1987.5	3260.3	2151.1
Bergaptol glucoside (**13**)	75.0	6.7	3.4	5.6	3.0	2.4	205.2	349.0	252.4
6′-*O*-Feruloylnodakenin (**14**)	22.2	43.1	14.6	91.0	95.8	28.8	728.4	1111.4	1131.2
Total	3056.3	2842.7	2311.7	2365.3	2776.3	879.9	5304.2	7887.2	5731.1

Sample I, the rhizome of *N. incisum*; sample II, Zhu-jie-qiang (竹節羌); sample III, Zhu-jie-qiang; sample IV, Can-qiang (蚕羌); sample V, Can-qiang; sample VI, Tiao-qiang (条羌); sample VII, the underground part of *N. forbesii*; sample VIII, Da-tou-qiang (大頭羌); sample IX, Da-tou-qiang. *a*) The content of each compound is indicated as weight (mg) per 100 g of the plant material. Each value is the mean of three determinations. *b*) Coefficient of variation (C.V.) is expressed by the following equation.

$$\text{C.V. (\%)} = \frac{\text{standard deviation (S.D.)}}{\text{mean value}} \times 100$$

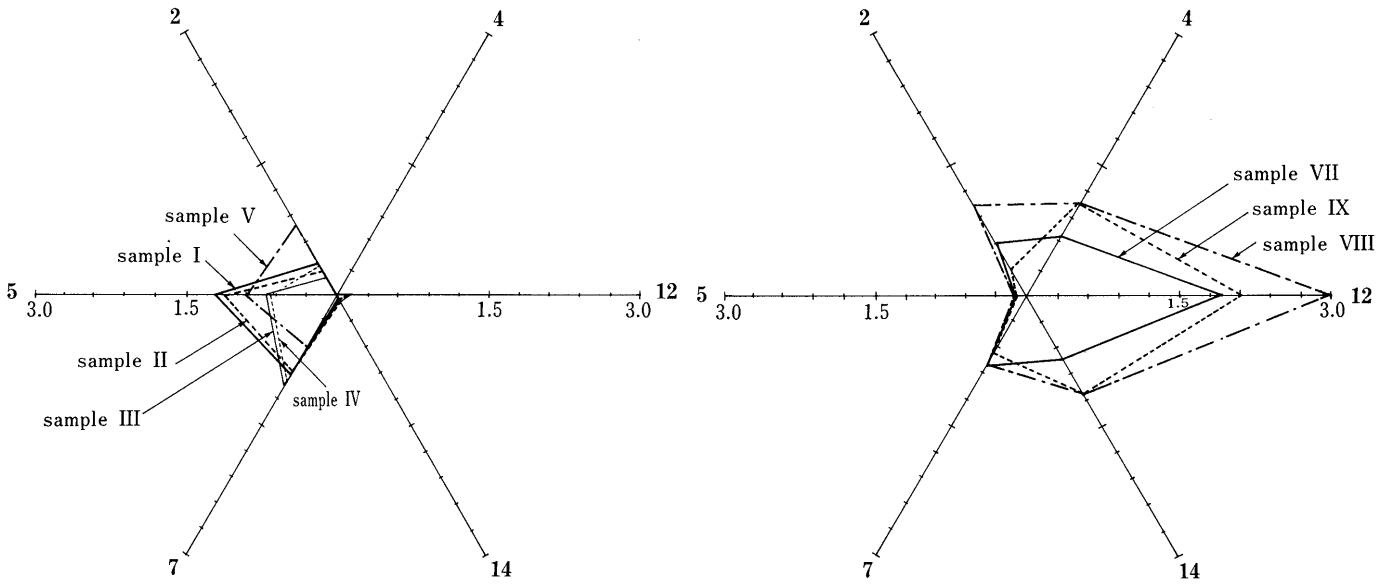

Fig. 3. Schematic Illustration of the Contents of Characteristic Constituents Present in the Drugs Derived from *N. incisum* (Group A, Left) and *N. forbesii* (Group B, Right)

Each axis represents the content (% (w/w)) of an indicated compound present in the plant material. Sample I, the underground part of *N. incisum*; samples II and III, Zhu-jie-qiang (竹節羌); samples IV and V, Can-qiang (蚕羌); sample VI, Tiao-qiang (条羌); sample VII, the underground part of *N. forbesii*; samples VIII and IX, Da-tou-qiang (大頭羌).

compositions of groups A and B (Fig. 3) were in accordance with those of *N. incisum* and *N. forbesii* (samples I and VII), respectively. In our previous paper,[5] we reported the identification of the botanical source of these commercially available crude drugs (samples II—VI and VIII—IX) by morphological and anatomical means; samples II—VI are derived from *N. incisum*, while samples VIII—IX from *N. forbesii*. Our present results well agreed with those classified by morphological and anatomical means.

Since the botanical source of Qiang-huo available in Chinese markets is either *N. incisum* or *N. forbesii*,[5] analysis of the constituents by HPLC is a useful method to distinguish the two botanical sources.

Experimental

Instruments Melting points were determined on a Yanagimoto micro-melting point apparatus. The following instruments were used for spectral measurement of isolated compounds: infrared (IR) spectra, Hitachi 260-10 infrared spectrometer; ultraviolet (UV) spectra, Shimadzu UV-210 digital double beam spectrophotometer; ^{1}H- and ^{13}C-NMR, JEOL GX-270 and JEOL FX 90Q spectrometers with tetramethylsilane as an internal standard; MS, JMS-DX 300 mass spectrometer; optical rotations, JASCO DIP-4 automatic polarimeter. Electron impact mass spectra (EI-MS) were measured at an ionization voltage of 70 eV and FAB-MS with glycerol as matrix. HPLC was carried out with a Shimadzu LC-4A liquid chromatograph equipped with a JASCO UVIDEC-100 UV spectrophotometer and a Shimadzu C-R6A chromatopac. Data were processed by an NEC PC-980 personal computer.

Materials Sample I is the underground part of *N. incisum*, collected at Ma-er-kang County (馬爾康県), Sichuan Province in May, 1988. Sample VII is the underground part of *N. forbesii*, collected in Huzhu County (互助県), Qinghai Province in September, 1988. Samples II (Zhu-jie-qiang; 竹節羌) and VI (Tiao-qiang; 条羌) were purchased from Cheng-du-522-zhong-yao-cai-cang-ku (成都522中薬材倉庫), Chengdu (成都), Sichuan Province in 1988; samples III (Zhu-jie-qiang; 竹節羌), IV (Can-qiang; 蚕羌) and VIII (Da-tou-qiang; 大頭羌) were purchased from Qing-hai-sheng-zhong-yao-cai-gong-si (青海省中薬材公司), Xining (西寧), Qinghai Province in 1988; samples V (Can-qiang; 蚕羌) and IX (Da-tou-qiang; 大頭羌) were obtained from Gan-su-sheng-zhong-yao-cai-gong-si (甘粛省中薬材公司), Lanzhou (蘭州), Gansu Province.

Column Chromatography Wako gel 100 (Wako Pure Chemical Industries Co., Osaka, Japan) was used for column chromatography. TLC plates (Merck Kieselgel 60 F_{254}; layer thickness, 0.25 mm) were purchased from E. Merck (Darmstadt, FRG). Amberlite IR-45 and Diaion HP-20 resins were obtained from Organo Co. (Tokyo) and Nippon Rensui Co. (Tokyo), respectively.

Extraction and Fractionation The dried and pulverized underground part of *N. forbesii* (5 kg) was extracted three times successively with Et_2O (15 l each) and EtOH (15 l each) at room temperature for 2 d (for each extraction). The Et_2O and EtOH solutions were concentrated *in vacuo* to give Et_2O and EtOH extracts, respectively, (450 and 270 g). The Et_2O extract was further partitioned between MeOH and hexane to give hexane-soluble (290 g) and MeOH-soluble (150 g) fractions. The MeOH-soluble fraction was applied to a column of silica gel (1.5 kg). The column was eluted successively with hexane, hexane–$CHCl_3$, $CHCl_3$, $CHCl_3$–EtOAc, EtOAc, EtOAc–MeOH to afford bergamottin (**1**, 25 mg), isoimperatorin (**2**, 5.35 g), cnidilin (**3**, 113 mg), *p*-hydroxyphenetyl anisate (**4**, 6.54 g), notopterol (**5**, 90 mg), bergapten (**6**, 23 mg), *trans*-ferulic acid (**7**, 1.2 g), demethylfuropinnarin (**8**, 43 mg), bergaptol (**9**, 8 mg), nodakenetin (**10**, 7 mg), β-sitosterol glucoside (**11**, 59 mg), nodakenin (**12**, 7.53 g) and bergaptol glucoside (**13**, 1.53 g). Similarly, the EtOH extract was subjected to column chromatography to give 6′-*O*-*trans*-feruloylnodakenin (**14**, 2.17 g).

Bergamottin (Bergaptin, 1) Colorless needles from hexane–Et_2O. mp 55—56 °C (lit.,[6] 59—61 °C). EI-MS *m/z*: 338 (M^+).

Isoimperatorin (2) Colorless prisms from EtOH. mp 109.5—110.5 °C. (lit.,[6] 109 or 108—110 °C). EI-MS *m/z*: 270 (M^+).

Cnidilin (3) Yellowish prisms from EtOAc–hexane. mp 112—113 °C. (lit.,[6] 117—118 or 115—115.5 °C). EI-MS *m/z*: 300 (M^+).

***p*-Hydroxyphenethyl Anisate (4)** Colorless prisms from $CHCl_3$–hexane. mp 129—131 °C (lit.,[3] 129—129.5 °C). EI-MS *m/z*: 272 (M^+).

Notopterol (5) Colorless needles from EtOAc–hexane. mp 90—92 °C (lit.,[3] 90—92 °C).

Bergapten (6) Colorless needles from EtOH. mp 187—188 °C. (lit.,[6] 188—191 °C). EI-MS *m/z*: 216 (M^+).

***trans*-Ferulic Acid (7)** Orthorhombic crystals from water. mp 173—174 °C. EI-MS *m/z*: 194 (M^+).

Demethylfuropinnarin (8) Yellow needles from EtOAc–Et_2O. mp 230—233 °C (lit.,[3] 231 °C). EI-MS *m/z*: 270 (M^+).

Bergaptol (9) EI-MS *m/z*: 202 (M^+). On methylation with diazomethane, **9** gave bergapten: colorless needles from EtOH, mp 187—188 °C.

Nodakenetin (10) Colorless needles from EtOAc–MeOH. mp 191—192 °C (lit.,[6] 185—192 °C). EI-MS *m/z*: 246 (M^+). $[\alpha]_D$ −26.0 (c=0.15, $CHCl_3$) (lit.,[6] $[\alpha]_D$ −25.4° ($CHCl_3$)).

β-Sitosterol Glucoside (11) Colorless plates, mp 302—304 °C. On hydrolysis with 10% HCl in MeOH, **11** gave β-sitosterol and glucose.

Nodakenin (12) Thin leaflets from EtOH. mp 217—219 °C (lit.,[6] 215—219 °C). $[\alpha]_D$ +24° (c=0.9, EtOH–H_2O (1:1)).

Bergaptol-*O*-β-D-glucopyranoside (13) Colorless needles from MeOH. mp 254—256 °C. FAB-MS (positive) m/z: 365 (M^++1), 277, 185 (M^+ – $C_6H_{12}O_6$), 115. UV λ_{max}^{MeOH} nm (log ε): 304 (2.34), 249 (2.71), 216 (2.72). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1700 (C=O), 1618, 1570, 1040. ^{1}H-NMR (270 MHz, C_5D_5N): δ 4.14—4.66 (6H, 2′,3′,4′,5,′6′,-H), 5.67 (1H, d, J=7.3 Hz, 1′-H), 6.28 (1H, d, J=9.8 Hz, 3-H), 7.33 (1H, s, 8-H), 7.76 (1H, m, 9-H), 7.81 (1H, d, J=2.0 Hz, 10-H), 8.64 (1H, d, J=9.8 Hz, 4-H). ^{13}C-NMR: see Table I.

Acid Hydrolysis of 13 A solution of **13** (10 mg) in 10% H_2SO_4 (5 ml) was heated for 40 min in a boiling water bath. The solution was passed through an Amberlite IR-45 column and the eluate was evaporated *in vacuo* to give a residue (8.5 mg). The residue was chromatographed on silica gel with MeOH–$CHCl_3$ (95:5) and MeOH–$CHCl_3$ (30:70) to give bergaptol (3.1 mg) and D-glucose (2.5 mg).

Enzymic Hydrolysis of 13 On treatment with β-glucosidase (10 mg, Sigma Chemical Co.) in acetate buffer (pH 5.0) for 48 h at 37 °C, **13** was converted to bergaptol (2.5 mg) and D-glucose (3 mg).

6′-*O*-*trans*-Feruloylnodakenin (14) Colorless needles from MeOH–H_2O. mp 138—140 °C. $[\alpha]_D$ +49.8° (c=0.5, EtOH–H_2O (1:1)). FAB-MS (negative) m/z: 583 (M^- – 1), 245 (nodakenetin – 1), 227 (245 – H_2O), 193 (ferulic acid – 1). UV λ_{max}^{MeOH} nm (log ε): 327 (2.84), 226 (2.51), 219 (2.45), 204 (2.91), λ_{sh} 248, 298. IR ν_{max}^{KBr} cm^{-1}: 3375 (OH), 1710 (ester C=O), 1620, 1590, 1565, 1505 (double bond, aromatic ring). ^{1}H-NMR (270 MHz, CD_3OD): coumarin moiety: δ 1.21, 1.41 (each 3H, each s, 12,13-H_3), 3.26 (2H, d, J=8.8 Hz, 9-H_2), 4.97 (1H, t, J=8.8 Hz, 10-H), 6.09 (1H, d, J=9.3 Hz, 3-H), 6.52 (1H, s, 8-H), 7.17 (1H, s, 5-H), 7.60 (1H, d, J=9.3 Hz, 4-H); sugar moiety: δ 3.18 (1H, dd, J=7.8, 9.0 Hz, 2′-H), 3.28 (1H, t, J=9.0 Hz, 4′-H), 3.42 (1H, t, J=9.0 Hz, 3′-H), 3.61 (1H, ddd, J=2.3, 8.0, 9.0 Hz, 5′-H), 4.35 (1H, dd, J=8.0, 11.8 Hz, 6′-H_a), 4.49 (1H, dd, J=2.3, 11.8 Hz, 6′-H_b), 4.62 (1H, d, J=7.8 Hz, 1′-H); ferulic acid moiety: δ 3.80 (3H, s, 3″-OMe), 6.17 (1H, d, J=16.1 Hz, 7″-H), 6.68 (1H, d, J=8.1 Hz, 5″-H), 6.79 (1H, dd, J=2.0, 8.1 Hz, 6″-H), 6.89 (1H, d, J=2.0 Hz, 2″-H), 7.46 (1H, d, J=16.1 Hz, 8″-H). ^{13}C-NMR: see Table I.

Acid Hydrolysis of 14 A solution of **14** (20 mg) in 10% H_2SO_4 (10 ml) was heated for 40 min in a boiling water bath. The solution was passed through an Amberlite IR-45 column and the eluate was evaporated *in vacuo* to give a residue (17 mg). The residue was applied to a column of silica gel and the column was eluted successively with $CHCl_3$–MeOH (95:5), $CHCl_3$–MeOH (90:10) and $CHCl_3$–MeOH (70:30) to give *trans*-ferulic acid (4.6 mg), nodakenetin (5.6 mg; $[\alpha]_D$ –23.7° (c=0.3, $CHCl_3$)) and D-glucose (4.2 mg).

Methanolysis of 14 A solution of **14** (15 mg) in 2% methanolic NaOMe (10 ml) was allowed to stand for 8 h at room temperature. After dilution with water, the reaction mixture was applied to a Diaion HP-20 column, which was eluted successively with water and MeOH. The MeOH eluate was concentrated *in vacuo* to give a residue. The residue was further chromatographed on silica gel with $CHCl_3$ and $CHCl_3$–MeOH (70:30) to give methyl *trans*-ferulate (4.1 mg) and nodakenin (9.5 mg; $[\alpha]_D$ +23.9° (c=0.7, EtOH–H_2O (1:1)).

Acetylation of 14 On acetylation with Ac_2O (1 ml) in pyridine (1 ml) at room temperature overnight, **14** gave a tetraacetate (**14a**, 5.5 mg): colorless needles from MeOH–H_2O (1:1), mp 110—112 °C. $[\alpha]_D$ +41.6° (c=0.5, EtOH–H_2O (1:1)) EI-MS m/z: 752 (M^+), 710 (M^+ – CH_3CO – H), 482, 229, 177. ^{1}H-NMR (270 MHz, $CDCl_3$): coumarin moiety: δ 1.26, 1.35 (each 3H, each s, 12,13-H_3), 3.17 (2H, d, J=8.8 Hz, 9-H_2), 4.80 (1H, t, J=8.8 Hz, 10-H), 6.16 (1H, d, J=9.5 Hz, 3-H), 6.65 (1H, s, 8-H), 7.12 (s, 5-H), 7.52 (1H, d, J=9.5 Hz, 4-H); sugar moiety: δ 1.86, 1.99, 2.05 (each 3H, each s, 2′,3′,4′-OAc), 3.81 (1H, m, 5′-H), 4.28 (1H, dd, J=6.1, 12.1 Hz, 6′-H_a), 4.35 (1H, dd, J=2.9, 12.1 Hz, 6′-H_b), 4.83 (1H, d, J=8.1 Hz, 1′-H), 4.94 (1H, dd, J=8.1, 9.5 Hz, 2′-H), 5.06 (1H, t, J=9.5 Hz, 4′-H), 5.24 (1H, t, J=9.5 Hz, 3′-H); ferulic acid moiety: δ 2.33 (3H, s, 4″-OAc), 3.85 (3H, s, 3″-OMe), 6.31 (1H, d, J=16.1 Hz, 7-H), 7.02 (2H, s, 5″,6″-H), 7.03 (1H, s, 2″-H), 7.60 (1H, d, J=16.1 Hz, 8-H); ^{13}C-NMR: see Table I.

Analysis of Crude Drug Extracts by HPLC HPLC was carried out under the following conditions: column, LichroCAT ODS (4 mm i.d. × 250 mm; E. Merck Co.); mobile phase, two solvent systems were used: MeCN–MeOH–H_2O (34:6:60) (solvent system A) for analysis of compounds **2**—**6**, **8** and **14**, and MeCN–MeOH–H_2O (21:6:73) (solvent B) for analysis of compounds, **7**, **9**, **10**, **12** and **13**; flow rate, 0.5 ml/min; detection, 254 nm.

Standard Curves An aliquot of the solution of each authentic compound was injected into an HPLC column, which was then eluted with solvent system A or B. A standard curve was plotted as peak-areas *versus* concentrations of the compound. Linear regression analysis was performed to determine the slope, intercept and correlation coefficient.

Recovery Experiment An exactly weighed authentic compound was dissolved in MeOH. An aliquot of the solution (*ca.* 1 μg/ml) was analyzed by HPLC. The net amount of added compound was calculated from the standard curve and the percentage recovery is shown in Table III.

Sample Preparation Each pulverized plant material (4.0 g) was extracted with MeOH (90 ml) for 2 h at 70 °C with a Soxhlet extractor. After being cooled, the MeOH solution was passed through MILLEX-HV (Millipore Product Division, Bedford, U.S.A.) and brought to a final volume of 80.0 ml. A portion (4 μl) of the solution was subjected to HPLC analysis.

References and Notes

1) J. Fan, S. Sun and S. Qin, *Zhongyao Tongbao*, **11**, 556 (1986).
2) Y. Sun and Y. Sun, *Zhongyao Tongbao*, **10**, 127 (1983).
3) M. Kozawa, M. Fukumoto, Y. Matsuyama and K. Baba, *Chem. Pharm. Bull.*, **31**, 2712 (1983).
4) H. Kohda and S. Motoyoshi, *Shoyakugaku Zasshi*, **37**, 165 (1983).
5) T. Namba, Z. Gu, T. Wang, M. Huo and K. Komatsu, *Shoyakugaku Zasshi*, in press (1991).
6) R. D. H. Murray, J. Mendez and S. A. Brown, "The Natural Coumarins. Occurrence, Chemistry and Biochemistry," John Wiley & Sons Ltd., Bristol, pp. 376 (bergapten and isoimperatorin), 378 (bergamottin), 429 (cnidilin) and 344 (nodakenetin and nodakenin).