Pure Appl. Chem., Vol. 74, No. 7, pp. 1199–1206, 2002.

Chemistry of phenolic compounds of licorice (*Glycyrrhiza* species) and their estrogenic and cytotoxic activities*

Taro Nomura[1], Toshio Fukai[1,‡], and Toshiyuki Akiyama[2]

[1]*School of Pharmaceutical Sciences, Toho University, 2-2-1 Miyama, Funabashi, Chiba 274-8510, Japan;* [2]*Exploratory Chemistry Research Laboratories, Sankyo Co., Ltd., 2-58 Hiromachi 1-Chome, Shinagawa-ku, Tokyo 140-8710, Japan*

Abstract: The genus *Glycyrrhiza* consists of about 30 species in which *G. glabra*, *G. uralensis*, *G. inflata*, *G. aspera*, *G. korshinskyi*, and *G. eurycarpa* are generally recognized as licorice because of their sweet taste. Except *G. korshinskyi*, we examined isoprenoid-substituted phenols of these licorices. Each plant could be characterized by some isoprenoid phenols. We also investigated the biological activities of the *Glycyrrhiza* phenols. In the course of screening phytoestrogen in medicinal plants, six *Glycyrrhiza* phenols exhibited the binding affinities for the bovine uterine estrogen receptor. The affinity of a dihydrostilbene with two 3-methyl-2-butenyl (prenyl) groups, gancaonin R, was higher than those of isoflavone phytoestrogens (genistein and daidzein) in dietary foods. The affinities of the other five phenols, a flavanone (liquiritigenin), two prenylflavanones (isobavachin and sigmoidin B), a prenylated coumestan (glycyrol), and a pyranoisoflav-3-ene (glabrene), were similar to that of the dietary isoflavone, genistein or daidzein. Cytotoxic activities of the *Glycyrrhiza* phenols against human oral tumor cell lines and HIV-infected MT-4 cells were also reviewed.

INTRODUCTION

Licorice (liquorice, kanzoh in Japanese, gancao in Chinese) is the name applied to the roots and stolons of some *Glycyrrhiza* species (Fabaceae) and has been used by human beings for at least 4000 years. The genus *Glycyrrhiza* consists of about 30 species, and chemical studies have so far been carried out on 15 of them. Glycyrrhizic acid is the major triterpenoid saponin in licorice root and the main sweetener of the herb. The saponin is used frequently as a tool for recognizing the herb and has been obtained from *G. glabra*, *G. uralensis*, *G. inflata*, *G. aspera*, *G. korshinskyi*, and *G. eurycarpa*, and thus, these plants are generally accepted as licorice [1]. In Japanese pharmacopeia, only *G. glabra* and *G. uralensis* are permitted to be used as licorice and licorice powder, and the other *Glycyrrhiza* species can be used as materials of licorice extract [2]. Following extraction, the herb yields the licorice products of commerce that are used as sweetening agents; flavoring for American-type tobaccos, chewing gums, candies, etc.; as a depigmentation agent in cosmetics; and as pharmaceutical products, e.g., antiulcer, antihepatitis medicines, antitussives, etc. Among them, the most important industrial use of the herb is in the production of additives as flavor and sweetening agents [1].

In the Far East, references to the effectiveness of licorice are contained in the *Shen Nong Ben Cao Jing*, the first Chinese dispensatory. In the Chinese book, 365 crude drugs are classified into three classes (upper: plants with lowest side-effects and nontoxic usefulness for health care; middle: plants

**Pure Appl. Chem.* Vol. 74, No. 7, 2002. A special topic issue on the science of sweeteners.
‡Corresponding author: E-mail: fukai@phar.toho-u.ac.jp

that are nontoxic or possess only weak toxicity in whose use care must be exercised; lower: toxic and only for clinical use). Licorice is described as belonging to the upper class and is recommended for lengthening one's life span, for improving health, for injury and swelling, and for its detoxification effect. One hundred and ten prescriptions are recorded in the earlier Chinese medicinal book *Shang Han Lun*, where 70 prescriptions include licorice. Some kinds of licorice have been imported into Japan. In the Japanese market, Chinese licorice is classified by its place of production, e.g., Northeastern licorice (Tohoku kanzoh in Japanese), Northwestern licorice (Seihoku kanzoh), Xinjiang licorice (Shinkyo kanzoh), etc. Among these licorice, Northeastern licorice had been identified as *G. uralensis*, but the original plants of the others had been unidentified. We investigated the phenolic constituents of certain *Glycyrrhiza* species identified by authorities, and many phenolic compounds were isolated from these plants [1]. In this paper, we describe the characteristic phenols of each *Glycyrrhiza* species and biological activities of some phenolic compounds.

Phenolic constituents of *Glycyrrhiza* species [1,3]

The main phenols of licorice are glycosides of liquiritigenin (4′,7-dihydroxyflavanone) and isoliquiritigenin (2′,4,4′-trihydroxychalcone), e.g., liquiritin, isoliquiritin, liquiritin apioside, licuraside, etc. [1]. As minor phenolic compounds, many isoprenoid-substituted flavonoids, chromenes, coumarins, dihydrostilbenes, and dihydrophenanthrenes were isolated from *Glycyrrhiza* species. Some of them characterized each plant [1,3].

Isoprenoid-substituted phenols of G. glabra

Three varieties of the species have been reported; Spanish licorice and Italian licorice are assigned to *G. glabra* var. *typica*, Russian licorice is *G. glabra* var. *glandulifera*, and Persian and Turkish licorices are *G. glabra* var. *violacea*. About 90 kinds of phenolic compounds have been isolated from the plants. About 50 of them are substituted with isoprenoid group(s), e.g., 3-methyl-2-butenyl (prenyl) group, 2,2-dimethylpyran ring, etc. These *G. glabra* could be classified into two groups with the constituents of isoprenoid-substituted flavonoids. Type I licorice is Spanish and Russian licorices. The main isoprenoid-substituted flavonoid of the plants is a pyranoisoflavan, glabridin (**1**). The 5-position of most flavonoids from the type I plants is unsubstituted, e.g., **1**, glabrene (**2**), glabrol (**3**), 3-hydroxyglabrol (**4**), etc. (Fig. 1). Type II licorice is Chinese and Kyrghiz *G. glabra*. From these plants, both 5-unsubstituted flavonoids (e.g., **2**) and 5-oxygenated flavonoids (e.g., 3′,8-diprenylated dalbergioidin, **5**), have been isolated. Nevertheless, most flavonoids from these plants are 5-hydroxy- or 5-methoxy-flavonoids. The main isoprenoid-substituted flavonoid of the Kirghiz licorice is compound **5**, but the isoflavan (**1**) has not been isolated [4].

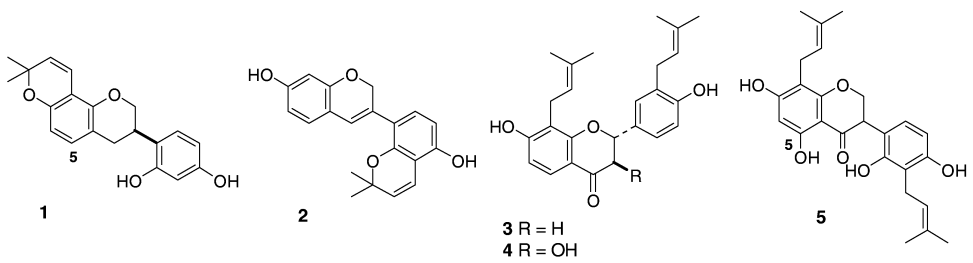

Fig. 1 Structures of isoprenoid-substituted flavonoids from *G. glabra*.

Isoprenoid-substituted phenols of G. uralensis

This licorice has been called Chinese licorice, Ural licorice, or Mongolian licorice. In Japan, *G. uralensis* is the most frequently used in clinical and called Northeastern licorice and Northwestern licorice by

its production place. About 100 kinds of phenols have been isolated from this licorice, in which about 70 compounds are isoprenoid-substituted phenols. The main isoprenoid-substituted flavonoids of the plant are isoflavans with two prenyl groups, licoricidin (**6**) and licorisoflavan A (**7**) (Fig. 2). Except licoricone (**8**), liconeolignan, glicoricone, and licofuranone, all isoprenoid-substituted flavonoids were substituted at the 5-position (1-position of pterocarpans and coumestans, 4-position of 2-arylbenzofurans) with an oxygen-functional group, e.g., compounds **6**, **7**, **9**, **10**, etc. As a characteristic flavonoid from Northwestern licorice, kumatakenin (**10**) was isolated, but not from Northeastern licorice [5].

Our group also studied the constituents of aerial part of this plant. The phenolic constituents from the aerial parts of the plants [e.g., gancaonins O (**11**), P (**12**), R (**13**), S (**14**), U (**15**), etc., Fig. 2] are different from those of underground parts. These dihydrostilbenes (**13** and **14**) and dihydrophenanthrene (**15**) are the characteristic compounds of the plant.

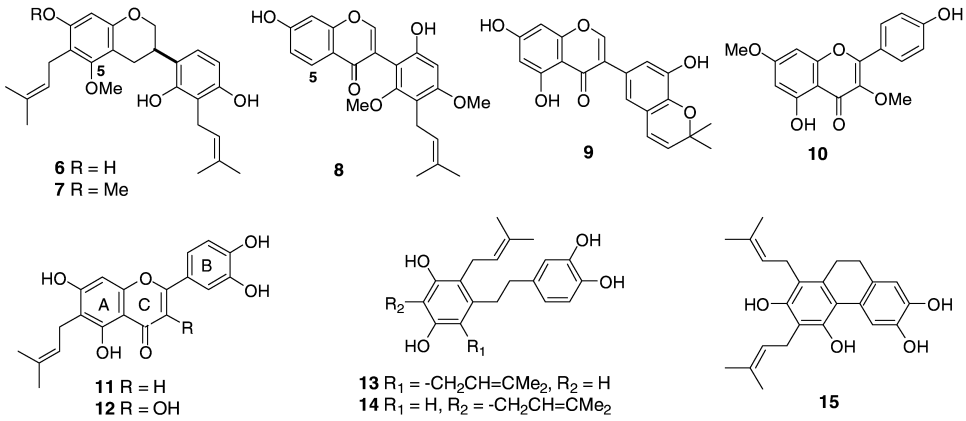

Fig. 2 Structures of flavonoids, dihydrostilbene, and dihydrophenanthrene from *G. ularensis*.

Isoprenoid-substituted phenols of G. inflata

G. inflata is the main species in Xinjiang licorice on Japanese market. In our morphological study of the licorice, it consisted of *G. inflata* (51 %), *G. eurycarpa* (8 %), *G. uralensis* (3 %), and some unidentified licorice (38 %). About 40 kinds of flavonoids have been isolated from *G. inflata*. Among them, 20 are isoprenoid-substituted flavonoids. The main isoprenoid-substituted flavonoid is licochalcone A (**16**). For this type of chalcone (e.g., **16**, **17**, etc., Fig. 3), the name "reversely constructed chalcone" or "retrochalcone" is frequently used*. The 5-position of most flavonoids of the plant, 2′- and 6′-positions of chalcones, is unsubstituted. Eight isoprenoid-substituted dibenzoylmethanes, i.e., glyinflanin A (**18**)–F, glycyrdione B, and 5′-prenyllicodione, were also isolated from the root of the plant as minor compounds. The ^{1}H- and ^{13}C-NMR spectra of these compounds are observed as an equilibrium mixture of tautomeric dibenzoylmethanes and β-hydroxychalcones in the solution (**18a** and **18b**, Fig. 3). In the solid state, these compounds may exist in the dibenzoylmethane form, because the ratio of two tautomers depends upon concentration of solution. Dibenzoylmethanes are well known in flavonoids chemistry as intermediates in flavone syntheses or in the isomerization "Wessely–Moser rearrangement"**. Glyinflanin A (**18**) was converted to 4′,7-dihydroxy-3′,6-diprenylflavone (prenyllicoflavone A) by heating in dry benzene.

*Dr. Shibata informed us that he wishes to change the name "retrochalcone" to "reversely constructed chalcone" based precisely on the biosynthetical scheme [1].

**The name reaction is the isomerization of flavones via C-ring opening but not rearrangement reaction.

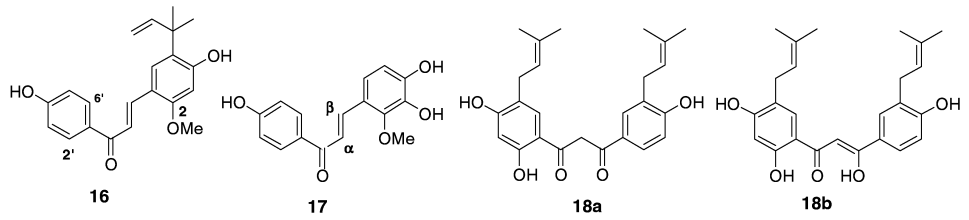

Fig. 3 Structures of licochalcones A (**16**) and B (**17**), and tautomeric structures (**18a** and **18b**) of glyinflanin A (**18**).

Isoprenoid-substituted phenols of G. eurycarpa

This licorice grows in northwest China and has been called yellow licorice. Previously, this plant had been assigned as *G. korshinskyi*. Recently, this licorice was recognized as a naturally occurring hybrid between *G. uralensis* and *G. inflata*, and thus, the species was named *G. eurycarpa* by Li [6]. Phenolic constituents of this plant resembled those of *G. inflata*, but not *G. uralensis*. About 40 phenolic compounds have been isolated from the underground parts of this species. Among them, 20 compounds are isoprenoid-substituted flavonoids. The main isoprenoid-substituted phenol is licochalcone A (**16**) as in *G. inflata*. Nevertheless, only three dibenzoylmethanes, glyinflanin B, kanzonol A, and 5′-prenyllicodione, were isolated from the plant.

Isoprenoid-substituted phenols of G. aspera

G. aspera is relatively unimportant in commerce because of a small plant. About 40 kinds of phenolic compounds have been isolated from the underground parts of this plant. Phenolic constituents resembled those of *G. uralensis*, e.g., licoisoflavone B (**19**), glycyrol (**20**), etc. The main isoprenoid-substituted phenols are licoricidin (**6**) and licorisoflavan A (**7**), as is the case with *G. uralensis*. These isoflavans were isolated from only these two *Glycyrrhiza* species. As an interesting compound, glyasperin E (**21**) was isolated (Fig. 4). This compound is a 3-oxygenated chromen-4-one derivative with a phenoxyl group and a prenyl group. Although 3-phenoxychromen-4-one derivatives have been synthesized, the compound (**21**) is the first example of natural product with such a skeleton.

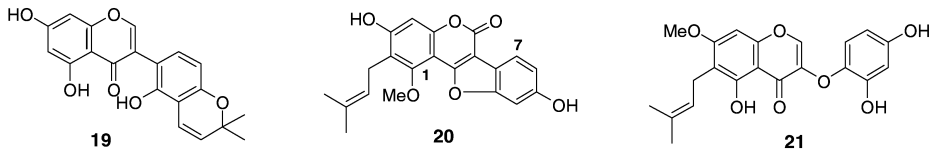

Fig. 4 Structures of licoisoflavone B (**19**), glycyrol (**20**), and glyasperin E (**21**).

From our chemical study of *Glycyrrhiza* species, it could be concluded that the origin of licorice, except *G. korshinskyi*, can be identified with the constituent of isoprenoid-substituted flavonoids*.

Biological activities of phenolic compounds of *Glycyrrhiza* species

Numerous pharmaceutical studies of licorice have been reported, as well as chemical studies of the plants [1]. Except for the effects against methicillin-resistant *Staphylococcus aureus* [7], anti-*Helicobacter pylori* activity [8], and the following bioactivities, Nomura and Fukai [1] and Shibata [9]

**G. korshinskyi* is widely distributed in the former Soviet Union, but the phenolic constituents of the licorice have not been reported.

reviewed the pharmaceutical studies of isolated phenols from *Glycyrrhiza* species. It may be an important study that aqueous solutions of some kinds of licorice saponins solubilize water-insoluble substances such as α-tocopherol and oleanolic acid [10]. This may be a restatement of a description of licorice in *Shen Nong Ben Cao Jing*: The oldest Chinese dispensatory also describes that licorice harmonizes a medicine with any drug. Generally, the traditional Chinese medicines consist of mixtures of crude drugs and require extraction with boiling water for lengthy periods. The solubilizing effect of the saponins is expected that Kampo-medicines (traditional Chinese medicine modified in Japan) contain lipophilic compounds when licorice is one of components of the drugs.

*Estrogen-like activity of prenylated phenols**

Traditional Japanese women with their high soy intake, a rich source of plant-derived estrogens (phytoestrogens), have a low incidence of breast cancer and few menopausal symptoms. This has led to the hypothesis that at menopause, phytoestrogens might act as natural selective estrogen-receptor modulators, tweaking estrogenic responses in the cardiovascular system, bone, and brain, but dampening responses in the breast and uterus. The finding that the soy-derived phytoestrogen genistein (4′,5,7-trihydroxyisoflavone) preferentially binds to the form of the estrogen receptor found mainly in the cardiovascular system lends some credence to that belief [11]. It is expected by much recent evidence that phytoestrogens exert beneficent actions on chronic diseases, e.g., heart attacks and other cardiovascular problems, osteoporosis, Alzheimer's disease, etc. [12]. Nevertheless, the isoflavonoids and lignans bind with low affinity to estrogen receptors, and thus, it is also suggested that they may induce production of sex hormone binding globulin in the liver and in this way influence sex hormone metabolism and biological effects [13]. On the other hand, there is another possibility of phytoestrogen sources in the Japanese lifestyle; most elder Japanese like Kampo-medicines rather than synthetic medicines. Furthermore, licorice is frequently used in Japanese over-the-counter (OTC) drugs that can be purchased without a doctor's prescription, e.g., stomachic, cough medicines, etc. (310 OTC drugs and 11 medicines in clinical use contain licorice extract [14]). Numerous phytoestrogens with a diversity of structures have now been recognized [12,15–19]. We examined whether other types of phenols with two or more aromatic rings bind to estrogen receptor. About 100 phenols from medicinal plants and their derivatives [1,20–23] were evaluated with estradiol receptor binding assay [15]. Among them, 13 compounds exhibited weak binding affinities ($1C_{50} < 1$ μg/mL) in which 6 compounds were isolated from *Glycyrrhiza* species. Relative binding affinity (RBA = [IC_{50} of 17β-estradiol (nM)]/[IC_{50} of test sample (nM)]) values are shown in Table 1. The affinity of gancaonin R (**13**) was stronger than those of genistein (RBA = 0.004) and daidzein (4′,7-dihydroxyisoflavone, RBA = 0.00035) [15]. The affinities of the other 12 compounds were similar to those of the isoflavones in dietary foods. We also studied the structure–function relationships with computation modeling of the bioactive phenols and the biological inactive compounds (Table 1). It has been considered that the binding sites at C-3 and C-17 of 17β-estradiol are rigid, but the lipophilic pocket near C-4–C-7 is flexible [24]. This was also indicated by our molecular modeling analyses.

*A part of this study was presented at the 21st IUPAC International Symposium on the Chemistry of Natural Products, Beijing, China, 11–16 October 1998; T. Fukai, T. Nomura, T. Akiyama, Abstract papers, p. 103.

Table 1 Binding affinities of phenolic compounds for the bovine uterine estrogen receptor.

Compound	RBA	Sources	Ref.
13	0.016	*G. uralensis* (aerial part)	[1]
8-Prenylkaempferol	0.0064	synthesis	[1]
8-Prenylquercetin	0.0057	synthesis	[1]
Isobavachin	0.0054	*G. pallidiflora* (root)	[1]
Isobavachalcone	0.0045	*M. cathayana* (root)	[20]
2	0.0022	*G. glabra* (root)	[1]
Tetrahydroglabrene	0.0016	synthesis	[1]
8-Geranylapigenin	0.0012	synthesis	[1]
20	0.0011	*G. uralensis* (underground part), *G. aspera* (root)	[1]
Albanol B	0.00095	*M. alba* (root bark)	[21]
Sigmoidin B	0.00077	*G. uralensis* (aerial part), *G. eurycarpa* (aerial part)	[1]
Sanggenon M	0.00048	*M. cathayana* (root bark)	[21]
Liquiritigenin	0.00038	*Glycyrrhiza* species (root and aerial part)	[1]

The binding affinities were measured as described previously [15]. IC_{50} value of 17ß-estradiol was 0.47–2.0 nM. IC_{50} of the following compounds were more than 0.2 μg/mL: *Glycyrrhiza* species (compounds in parenthesis were derived from natural products) [1], afromosin, calycosin, eudiol, medicarpin, 1-methoxyphaseollidin, gancaonins B, C, E, G, K, O (**11**), S (**14**), U (**15**), V, glabranin, glabridin (**1**), glabrol (**3**), glabrone, glyasperins C, D, glycycoumarin, glyinflanin C, isoglycyrol, kanzonol X, kumatakenin (**10**), licochalcones A (**16**), B (**17**), licoisoflavanone, licoisoflavones A, B (**19**), licoricidin (**6**), licoricone (**8**), ovaliflavanone B, paratocarpin L, pinocmbrin, 7-*O*-methylglabranin, 3′-prenylkievitone, 6-prenylpinocembrin, semilicoisoflavone B (**9**), topazolin, (gancaonin U tetramethyl ether, licoricidin triacetate, tetrahydrolicoricidin), *Morus* species [1,21], carpachromene, cyclomorusin, kuwanons C, E, H, M, S, moracins C, P, morusin, mulberrofurans B, G, oxydihydromorusin, sanggenol C, sanggenon A (morusinhydroperoxide, compound A, morusin diacetate, kuwanon E triacetate, kuwanon G octamethyl-d_3 ether), *Artocarpus* species [21,22], artocarpesin, artonins E, G, J, L, cycloartocarpin, cyclohetrophyllin, norartocarpetin, *Broussonetia* species [1,21], broussoflavonols B, C, kazinols B, E, F, N, *Cudrania* species [21], cudraflavone A, *Antiaris* species [21], antiarones F, H, I, J, K, sigmoidin A, *Epimedium* species [23], ikarisosides A, B, C, E, F. The details of the affinities of these compounds (e.g., inhibition percentage at 1 μM) are available by the corresponding author (TF).

Cytotoxic activities of G. phenols

Prescreening of bioactive compounds with Rec-assay

Rec-assay was developed for screening chemical and environmental mutagens. Recombinationless mutant cells of *Bacillus subtilis* (M45) are more sensitive to the cell-killing action of chemical mutagens, e.g., mytomycin C, *N*-nitroso-*N*-methylurethane, etc., than are the wild-type bacteria (H17). The assay is also useful as prescreening of anticancer drugs such as dynemicines. Since the sensitivity of the rec-assay to chemicals having induction activity of DNA damage is higher than other screening systems, this method may be useful as prescreening of bioactive compounds in crude drugs as well as in microorganisms. We tried the evaluation of the rec-assay for detection of bioactive compounds in phenolic compounds obtained from licorice. Sixty-nine compounds in 108 *Glycyrrhiza* phenols showed the inhibitory activity for the growth of both *B. subtilis* H17 and M45. Among the active compounds, 7 compounds, isoliquiritigenin, semilicoisoflavone B (**9**), gancaonin C [6-(*E*)-3-hydroxymethyl-2-butenylgenistein], 6- and 8-prenylated eriodictyols, licoisoflavone B (**19**), and licoisoflavanone (2,3-dihydrolicoisoflavone B), showed positive to rec-assay [25].

Cytotoxic activity against human oral tumor cell lines

Only a few studies of flavonoids in dentistry, except the study of cariogenic bacterium, have been reported. We have initiated a series of studies on the interaction between polyphenols and the oral environment. Glyasperin A (3′,6-diprenylated kaempferol, CC_{50} < 0.008 mM), 1-methoxyficifolinol (2,8-diprenyl-3,9-dihydroxy-1-methoxypterocarpan, CC_{50} = 0.012 mM), gancaonin P (**12**, 0.014 mM), licochalcone B (**17**, 0.018 mM), topazolin (6-prenylated 3-*O*-methylkaempferol, 0.019 mM), gancaonin O (**11**, 0.020 mM), and gancaonin R (**13**, 0.021 mM), showed relatively higher cytotoxic activ-

ity against human oral squamous cell carcinoma cell line HSC-2. Human salivary gland tumor cell line HSG showed comparable sensitivity against these phenols, whereas normal human gingival fibroblast HGF was much more resistant. This suggests that these compounds display specific cytotoxic activity against cancer cell lines rather than normal cells. However, further systematic studies with many normal and tumor cells are necessary to confirm this point [26,27].

Anti-HIV activity

Most of the *Glycyrrhiza* phenols reduced the viable cell number of mock-infected and HIV-infected MT-4 cells to comparable extents, yielding no significant anti-HIV activity; selective index (SI = [CC_{50} for MT-4 cells]/[EC_{50} for HIV-infected MT-4 cells]) < 10. However, several compounds, e.g., 3-hydroxyglabrol (**4**, SI = 10) and kumatakenin (**10**, SI = 20), showed low anti-HIV activity [27].

REFERENCES

1. T. Nomura and T. Fukai. In *Progress in the Chemistry of Organic Natural Products,* Vol. 73, W. Herz, G. W. Kirby, R. E. Moore, W. Steglich, Ch. Tamm (Eds.), pp. 1–140, Springer, Vienna (1998) and references cited therein.
2. *The Japanese Pharmacopeia*, 14th ed., pp. 839–841, Ministry of Health, Labour and Welfare, Tokyo, Japan (2001).
3. S. Shibata and T. Saitoh. *J. Indian Chem. Soc*. **55**, 1184 (1978).
4. T. Fukai, B.-S. Cai, T. Nomura. *Nat. Med*. **55**, 311 (2001).
5. S. Shibata and T. Hiraga. *Pharm. Tech. Jpn*. **2**, 569 (1986).
6. P.-C. Li. *Xi Bei Zhi Wu Yan Jiu (Act. Bot. Bor.-Occ. Sinica, China)* **4**, 117 (1984).
7. (a) T. Hatano, Y. Shintani, Y. Aga, S. Shiota, T. Tsuchiya, T. Yoshida. *Chem. Pharm. Bull.* **48**, 1286 (2000); (b) T. Fukai, A. Marumo, K. Kaitou, T. Kanda, S. Terada, T. Nomura. *Fitoterapia*. In press.
8. T. Fukai, A. Marumo, K. Kaitou, T. Kanda, S. Terada, T. Nomura. *Life Sci.* **71**, 1449 (2002).
9. S. Shibata. *Yakugaku Zasshi* **120**, 849 (2000).
10. Y. Sasaki, K. Mizutani, R. Kasai, O. Tanaka. *Chem. Pharm. Bull*. **36**, 3491 (1988).
11. E. Finkel. *Lancet* **352**, 1762 (1998).
12. M. Kitaoka, H. Kadokawa, M. Sugano, K. Ichikawa, M. Taki, S. Takaishi, Y. Iijima, S. Tsutsumi, M. Boriboon, T. Akiyama. *Planta Med*. **64**, 511 (1998).
13. A. M. Pino, L. E. Valladares, M. A. Palma, A. M. Mancilla, M. Yáñez, C. Albala. *J. Clin. Endocrinol. Metab*. **85**, 2797 (2000).
14. Japan Pharmaceutical Information Center (Ed.), *Drugs in Japan, Data Base, April 2001* [CD-ROM], JPIC/Jiho, Tokyo, Japan (2001).
15. K. Ichikawa, M. Kitaoka, M. Taki, S. Takaishi, Y. Iijima, M. Boriboon, T. Akiyama. *Planta Med*. **63**, 540 (1997).
16. M. Miyamoto, Y. Matsushita, A. Kiyokawa, C. Fukuda, Y. Iijima, M. Sugano, T. Akiyama. *Planta Med*. **64**, 516 (1998).
17. K. Masuda, T. Akiyama, M. Taki, S. Takaishi, Y. Iijima, M. Yamazaki, N. Aimi, J. Jato, P. G. Waterman. *Planta Med*. **66**, 169 (2000).
18. E. Minami, M. Taki, S. Takaishi, Y. Iijima, S. Tsutsumi, T. Akiyama. *Chem. Pharm. Bull*. **48**, 389 (2000).
19. S. Nishimura, M. Taki, S. Takaishi, Y. Iijima, T. Akiyama. *Chem. Pharm. Bull*. **48**, 505 (2000).
20. T. Fukai, Y.-H. Pei, T. Nomura, C.-Q. Xu, L.-J. Wu, Y.-J. Chen. *Heterocycles* **43**, 425 (1996).
21. T. Nomura. In *Progress in the Chemistry of Organic Natural Products*, Vol. 53, W. Herz, H. Grisebach, G. W. Kirby, Ch. Tamm (Eds.), pp. 87–201, Springer, Vienna (1988) and references cited therein.
22. T. Nomura and Y. Hano. *Nat. Prod. Rep*. **11**, 205 (1994) and references cited therein.

23. T. Fukai and T. Nomura. *Phytochemistry* **27**, 259 (1988).
24. P. R. Kym, G. M. Anstead, K. G. Pinney, S. R. Wilson, J. A. Katzenellenbogen. *J. Med. Chem*. **36**, 3910 (1993).
25. T. Fukai, B.-S. Cai, K. Maruno, Y. Miyakawa, M. Konishi, T. Nomura. *Phytochemistry* **49**, 2005 (1998).
26. H. Sakagami, Y. Jiang, K. Kusama, T. Atsumi, T. Ueha, M. Toguchi, I. Iwakura, K. Satoh, T. Fukai, T. Nomura. *Anticancer Res.* **20**, 271 (2000).
27. T. Fukai, H. Sakagami, M. Toguchi, F. Takayama, I. Iwakura, T. Atsumi, T. Ueha, H. Nakashima, T. Nomura. *Anticancer Res.* **20**, 2525 (2000).