

STUDIES ON THE CONSTITUENTS OF THE SEEDS OF *VACCARIA*
SEGETALIS

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Abstract—*Vaccaria segetalis* is an annual herb widely distributed in Asia, Europe and North America. The seeds of this plant, known as Wang-Bu-Liu-Xing in traditional Chinese medicine, have been widely used to cure the diseases associated with women after giving birth. A wide range of chemical compounds including triterpene saponins, alkaloids, cyclic peptide, phenolic acid, flavonoids, and steroids have been isolated from the seeds of this herb. A comprehensive account of the chemical constituents and the biological activities are presented in this review.

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1. Introduction

The plant *Vaccaria segetalis* (Neck.) Garcke (syn. *V. pyramidata* Medik) (Caryophyllaceae) is an annual herb widely distributed in Asia, Europe and North America. In Japan, it has been cultivated as a garden plant for several centuries, and in China it is distributed all over the country, except southern China. The seeds of this plant, known as Wang-Bu-Liu-Xing in traditional Chinese medicine, have been widely used to cure the diseases associated with women after giving birth. According to traditional Chinese medicine, Wang-Bu-Liu-Xing has the capacity to promote diuresis and milk secretion, activate blood circulation and relieve carbuncle.¹ It is also used in the treatment of amenorrhea and breast infections.¹ Previous studies on the seeds of this species led to the isolation of several triterpenoid saponins,²⁻⁷ seven cyclic peptides⁸⁻¹¹ and one flavonoid (vacarin).¹² Recently, our research group re-investigated the components of *V. segetalis*. This review mainly discusses the isolation, classification and biological activities of the components isolated by our research group from this species.

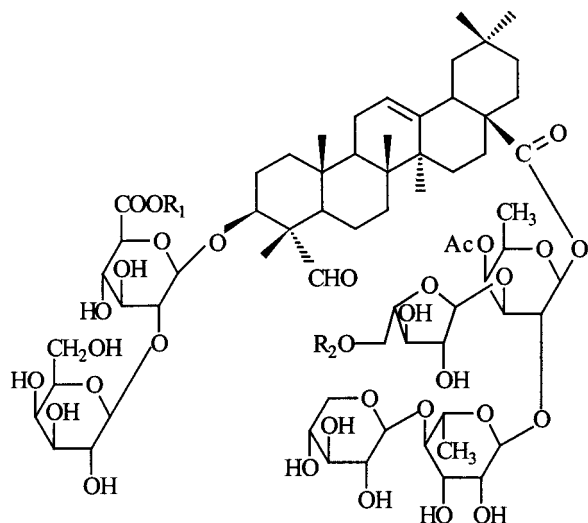
2. Phytochemistry

The powdered seeds of *V. segetalis* (50 Kg) were extracted successively with petroleum ether (two times) and 95% EtOH (three times). After evaporation of ethanol *in vacuo*, the residue was suspended in water and then extracted successively with CH₂Cl₂, EtOAc and *n*-BuOH. Forty-five compounds were isolated from these fractions. According to their structures, they can be classified as: triterpenoid saponins, alkaloids, cyclic peptides, phenolic acid, flavonoids, steroids, and other compounds.

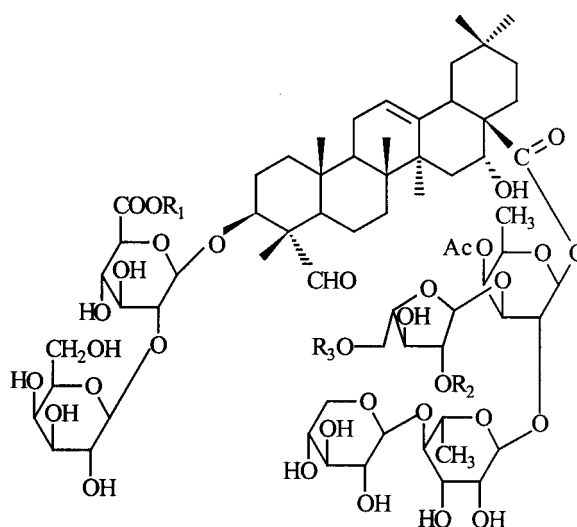
2.1 Triterpene saponins

Research has shown that saponins from a variety of sources, such as medicinal plants and foodstuffs, have considerable health benefits.^{13,14} Saponins occurring in *V. segetalis* seeds are glycosides of triterpenes. Triterpene aglycons can be substituted with several functional groups that result in a number of structurally different aglycons. In the triterpene saponins that we isolated from this seed, these aglycons have received trivial names such as gypsogenin, quillaic acid, gypsogenic acid, 16-hydroxygypsogenic acid, 3,4-secogypsogenic acid and oleanolic acid. The aglycons can be further substituted with 3-6 sugars, including glucopyranose, xylopyranose, arabinofuranose, fucopyranose, galactopyranose, glucuronopyranosic acid and rhamnopyranose. Twenty three triterpene saponins were isolated from the butanol fraction by repeated column chromatography on Diaion HP-20, silica gel and RP-18 silica gel, including six gypsogenin type saponins (**1-6**), six quillaic acid type saponins (**7-12**), seven gypsogenic acid type saponins (**13-19**), one 16-hydroxygypsogenic acid type saponin (**20**), two

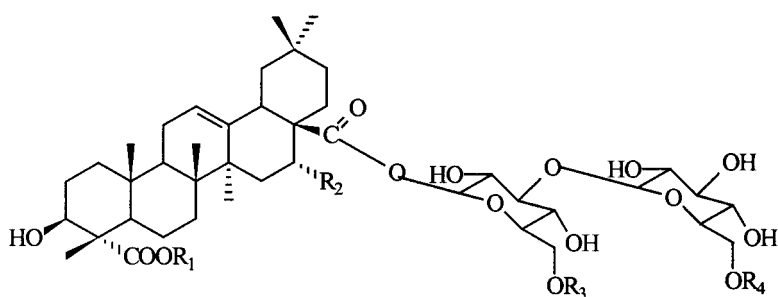
3,4-*seco* derivative of gypsogenic acid type saponins (**21**, **22**)¹⁵⁻²⁰ and one oleanolic acid type saponin. Fourteen of them (**2-11**, **15**, **18**, **19** and **23**) are new compounds.

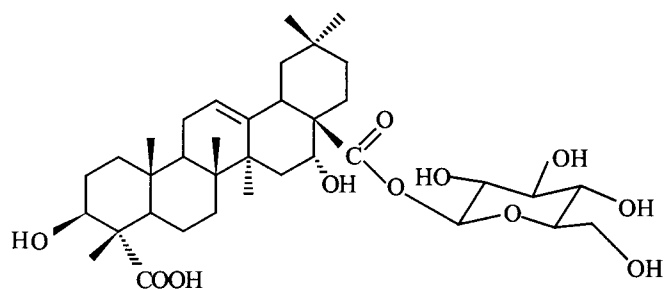
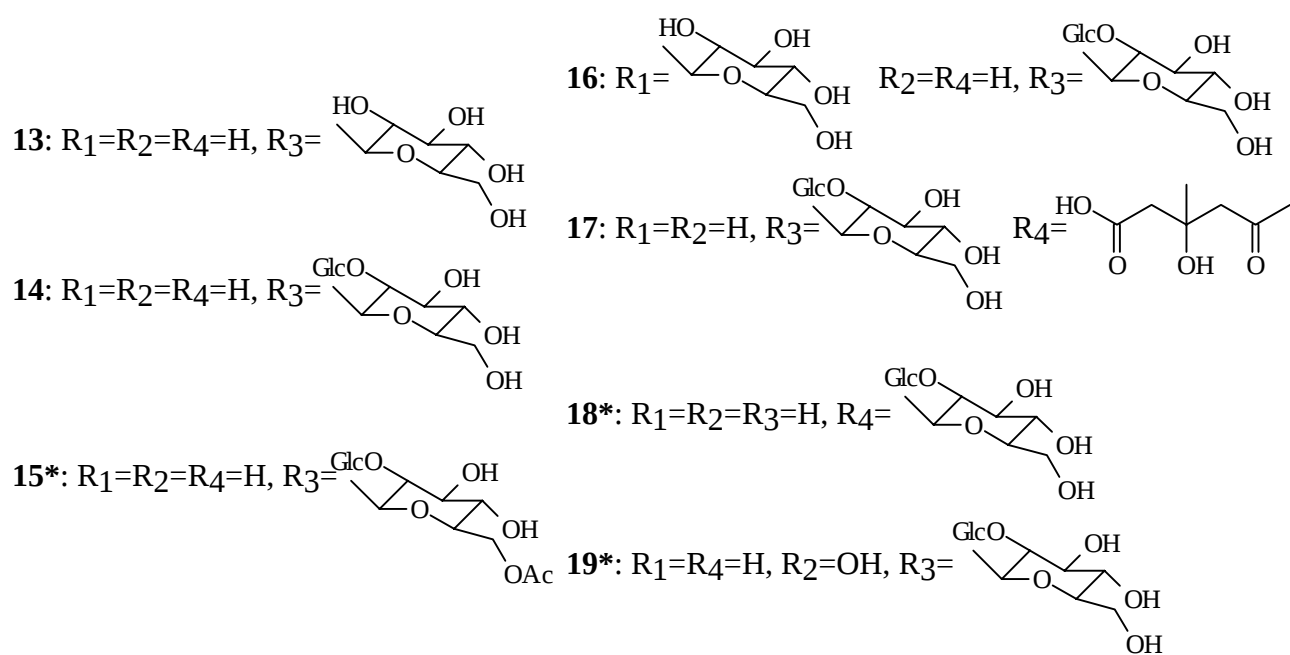


- 1** : $R_1=R_2=H$
2*: $R_1=H$, $R_2=Ac$
3*: $R_1=CH_3$, $R_2=Ac$
4*: $R_1=CH_3$, $R_2=H$
5*: $R_1=CH_2CH_3$, $R_2=H$
6*: $R_1=CH_2CH_2CH_2CH_3$, $R_2=H$

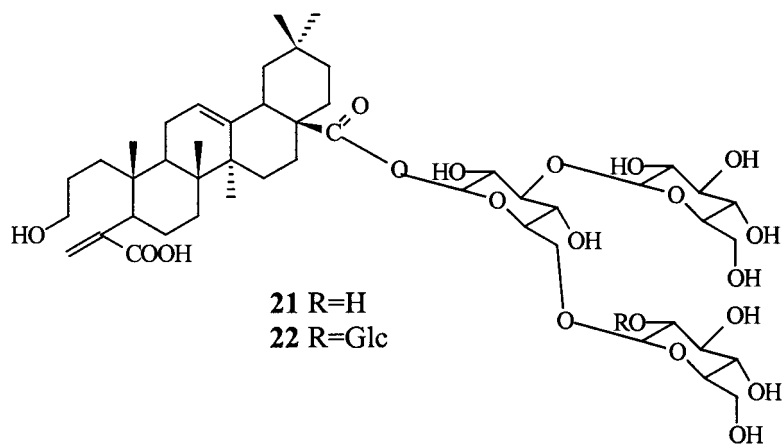


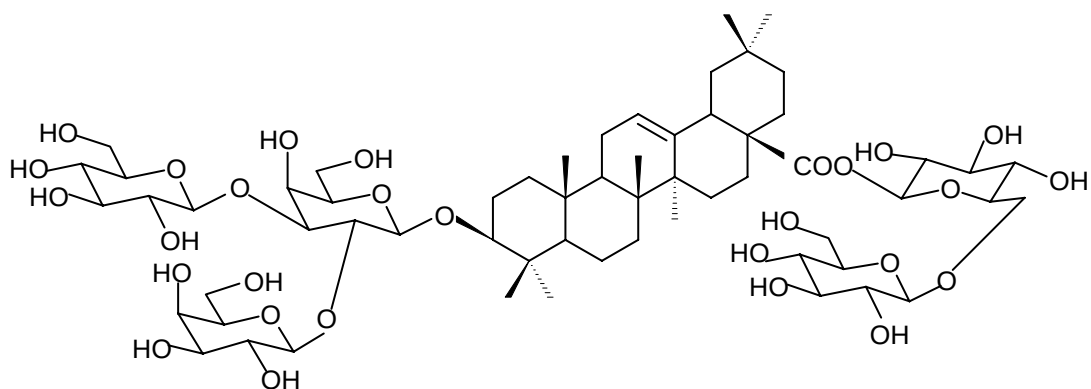
- 7***: $R_1=R_2=H$ $R_3=Ac$
8*: $R_1=H$, $R_2=R_3=Ac$
9*: $R_1=CH_3$, $R_2=R_3=Ac$
10*: $R_1=CH_3$, $R_2=H$, $R_3=Ac$
11*: $R_1=CH_2CH_2CH_2CH_3$, $R_2=H$, $R_3=Ac$
12 : $R_1=R_2=R_3=H$





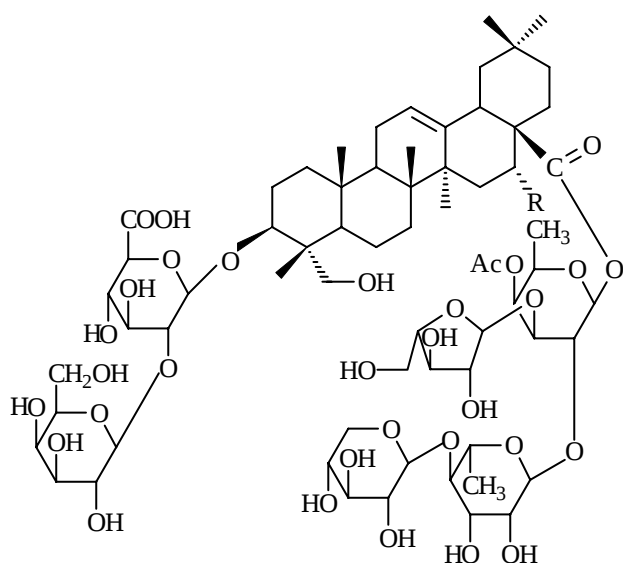
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23*

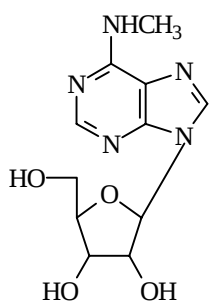
In addition to the triterpenoid saponins that we present here, there are two more compounds (vaccarosides **F** and **H**) reported by Nikaido *et al.* from this seed.⁷



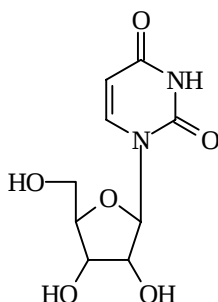
Vaccaroside **F**: R=H
Vaccaroside **H**: R=OH

2.2 Alkaloids

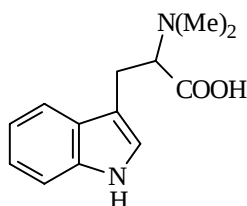
Four alkaloids were isolated from the butanol fraction and the ethyl acetate fraction and identified as 6-*N*-methyl adenosine (**24**), *N*, *N*-dimethyl-*L*-tryptophan (**26**) (from 30% butanol fraction), uridine (**25**) and allantoin (**27**) (from ethyl acetate fraction).²¹⁻²³ It is well known that allantoin has been used topically to stimulate healing of suppurating wounds and resistant ulcers.²⁴



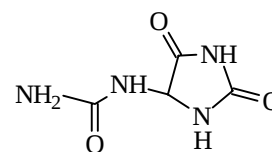
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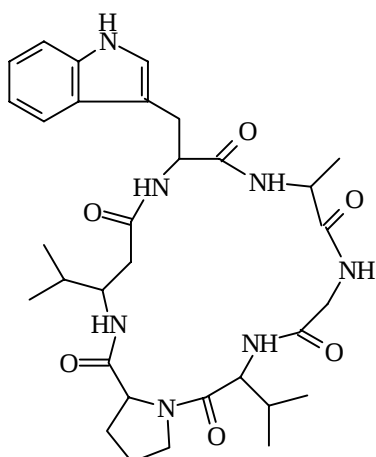
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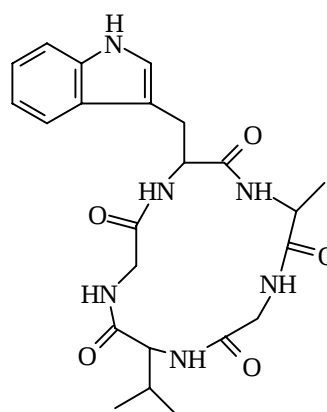
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2.3 Cyclic peptides

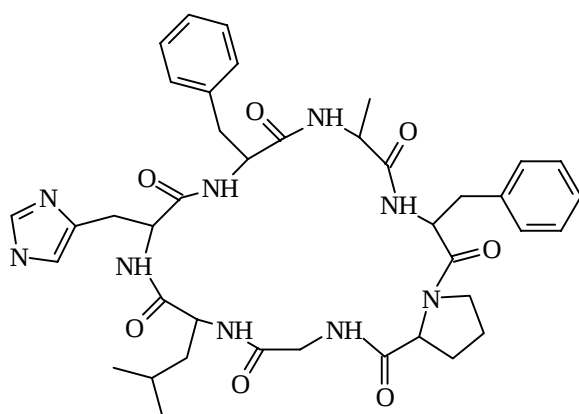
Cyclic peptides are natural products exhibiting a wide variety of biological functions. Large numbers of cyclic peptides with unique structures and various pharmacological activities are reported from marine and microorganisms,²⁵ whereas only few examples are known from higher plants.²⁶⁻³⁰ During our research four cyclic peptides were isolated from the ethyl acetate fraction. Compound (**28**) is a cyclic hexapeptide, and **29** is a cyclic pentapeptide, and **30** and **31** are cyclic heptapeptides. These compounds



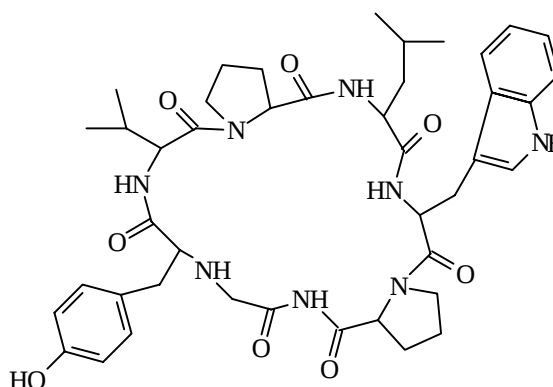
28: Segetalin A



29: Segetalin B

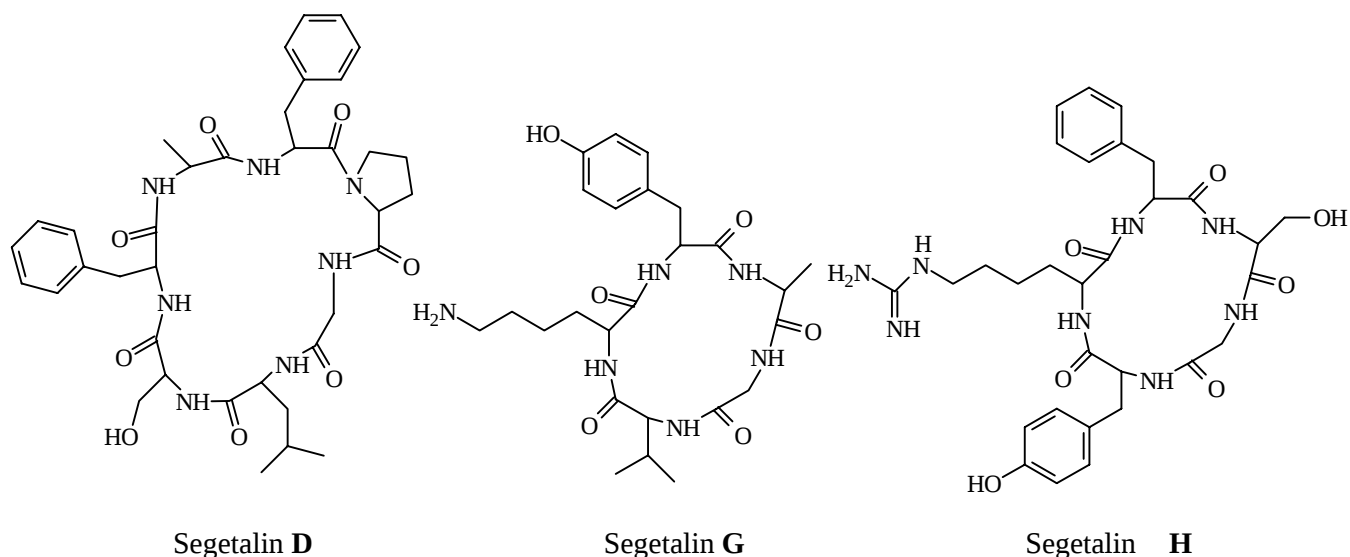


30: Segetalin C



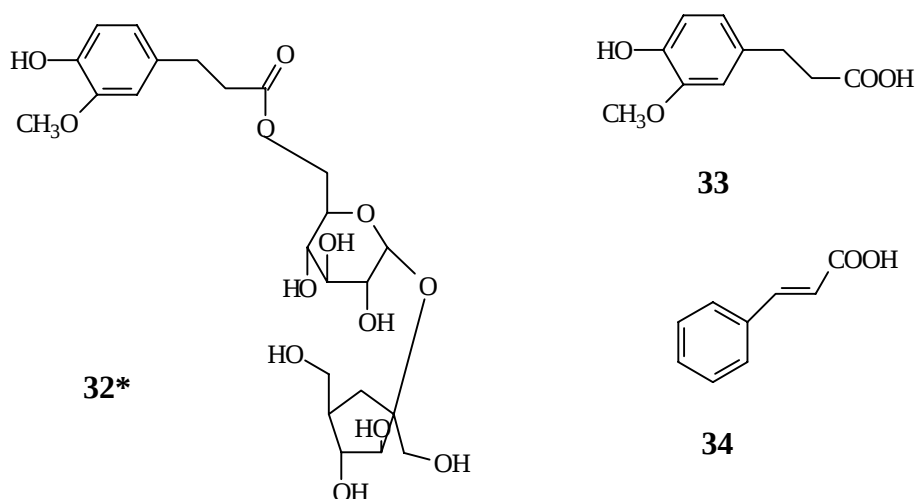
31: Segetalin E

were first reported by Itokawa *et al.*, who reported seven cyclic peptides (segetalins **A-E**, **G-H**) from this species.⁸⁻¹¹ Among these, segetalins **A** (**28**), **B** (**29**), **G** and **H** showed estrogen-like activity assayed by the increment of uterus in ovariectomized rats.³¹



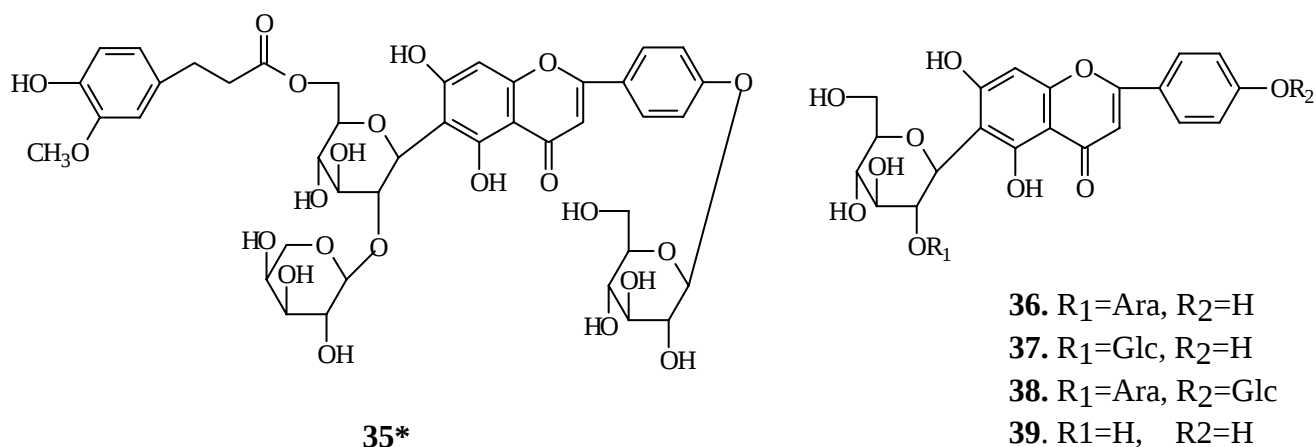
2.4 Phenolic acids

Phenolic acids have received considerable attention as potential protective factors against cancer and heart diseases in part because of their potent antioxidative properties.^{32, 33} Phenolic acids are hydroxylated derivatives of benzoic and cinnamic acids. One new phenylpropanoid glycoside (**32**)²¹ and two known free phenolic acids, dihydroferulic acid (**33**)²² and *E*-3-phenylacrylic acid (**34**),³² were isolated from the ethyl acetate fraction of this seed.



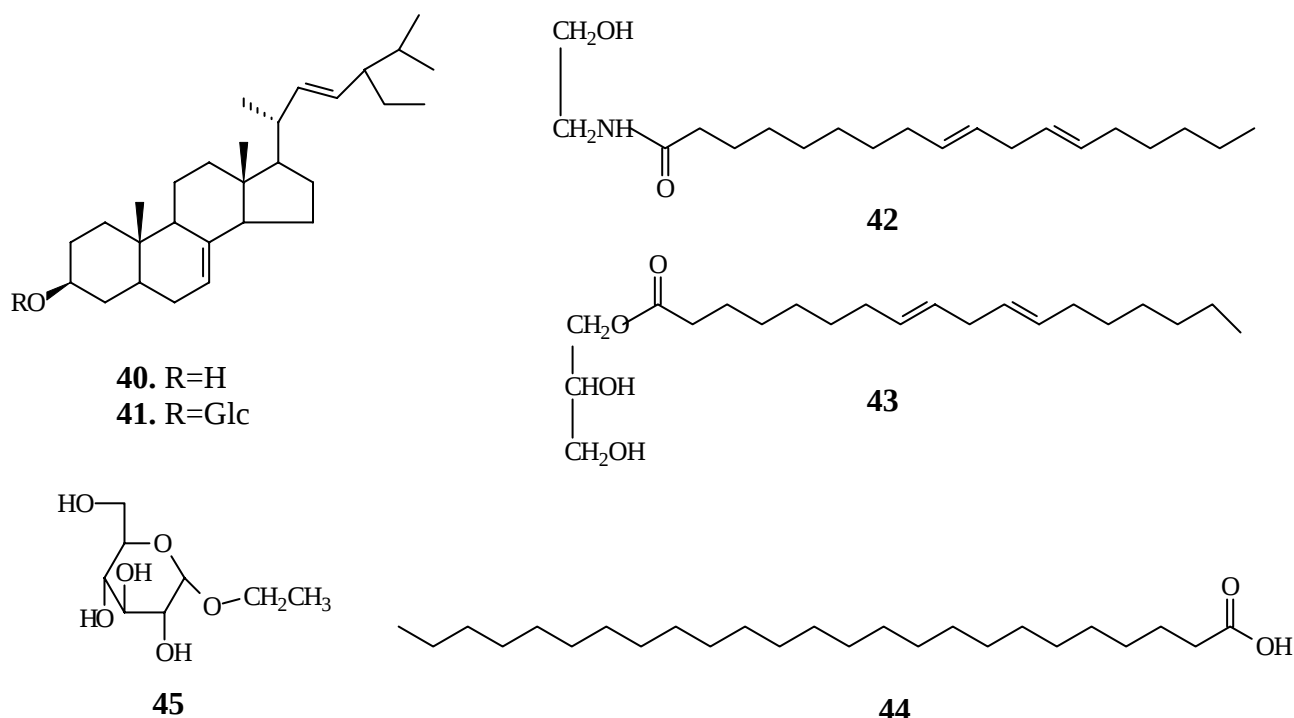
2.5 Flavonoids

Flavonoids occur naturally in the plant kingdom and are widely contained in the human diet. These flavonoids have shown many biological and pharmacological activities, such as antioxidant activity,³³⁻³⁵ tumor growth-inhibitory activity in various cancer cell lines *in vitro*,³⁶⁻³⁸ and reducing activity against the risk of the breast cancer.^{39, 40} Five flavonoids were isolated from the *n*-butanol fraction. They have the same aglycon, apigenin, a common flavone. Among them, compound (35) is a new compound that has a dihydroferuloyl group at position 6 of the glucosyl group.⁴¹ Compound (38) is vacarin that has been reported by the previous researchers.^{12,42}



2.6 Steroids

Two very common steroids, stigmast-7,22-dien-3-ol (**40**) and its glucoside (**41**) were also isolated from the methylene chloride fraction of this species.³²



2.7 Others

Compound (42) is an amide,³² which is a new natural product. Compound (43) is a monoglyceride. Compound (44) is a fatty acid and compound (45) is ethyl- α -glucoside.

3. Bioactivities

In order to find the bioactive compounds that are responsible for the traditional use of this seed, two bioassays, inhibitory effect on the growth of the HL-60 cell line and growth-inhibitory activity on luteal cells of rats, were used. One new flavonoid (35) exerted a weak inhibitory effect on the growth of the HL-60 cell line with an IC₅₀ value of 97.4 μ g/mL. Eighteen compounds were tested for the growth-inhibitory activity on luteal cells of rats. Among them, three new triterpenoids (compounds 4, 10 and 11) exhibited strong inhibitory effect on the growth of luteal cells of rats resulting in 100% at a concentration of 20 μ g/mL. It was known that steroid saponins have the activity of inhibition of the growth of luteal cells.⁴³ These compounds are unique examples of triterpenoid saponins that show growth-inhibitory activity on luteal cells.

4. Conclusion

In conclusion, Forty-five compounds were isolated from the seeds of *V. segetalis* and identified by our research group. Sixteen of them are new compounds, one of them is new natural product. Most of these new compounds' structures are complex. New 2D-NMR techniques, HMQC-TOCSY and HMBC-TOCSY, were applied to determine the structures of these new triterpenoid saponins. Eighteen compounds were tested for the growth-inhibitory activity on luteal cells of rats. Three new compounds showed very strong inhibition resulting in 100% at a concentration of 20 μ g/mL. One new flavonoid showed inhibitory effect on the growth of the HL-60 cell line.

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REFERENCES

1. Jiangsu New Medical College, *Zhong-yao-da-ci-dian*, Shanghai Science and Technology Publisher, 1986; p. 311.
2. V. I. Litvinenko, K. Amanmuradov, and N. K. Abubakirov, *Khim. Prir. Soed.*, 1967, **3**, 159.
3. K. Amanmuradov and N. K. Abubakirov, *Khim. Geol. Nauk.*, 1964, **6**, 104.
4. H. Morita, Y. S. Yun, K. Takeya, H. Itokawa, K. Yamada, and O. Shiota, *Bioorg. Med. Chem. Lett.*, 1977, **7**, 1095

5. Y. S. Yun, K. Shimizu, H. Morita, K. Takeya, H. Itokawa, and O. Shirota, *Phytochemistry*, 1998, **47**, 143.
6. K. Koike, Z. H. Jia, and T. Nikaido, *Phytochemistry*, 1998, **47**, 1343.
7. Z. H. Jia, K. Koike, M. Kudo, H. Y. Li, and T. Nikaido, *Phytochemistry*, 1998, **48**, 529.
8. H. Morita, Y. S. Yun, K. Takeya, and H. Itokawa, *Tetrahedron*, 1995, **51**, 5987.
9. H. Morita, Y. S. Yun, K. Takeya, and H. Itokawa, *Tetrahedron*, 1995, **51**, 6003.
10. H. Morita, Y. S. Yun, K. Takeya, and H. Itokawa, *Phytochemistry*, 1996, **42**, 439.
11. Y. S. Yun, H. Morita, K. Takeya, and H. Itokawa, *J. Nat. Prod.*, 1997, **60**, 216.
12. R. T. Baeva, M. O. Karryev, V. I. Litvinenko, and N. K. Abubakirov, *Khim. Prir. Soedin.*, 1974, **10**, 1714.
13. N. Rao and D. M. Gurfinkel, *Drug Metabol. Drug Interact.*, 2000, **17**, 211.
14. K. Hostettmann, and A. Marston, *Saponins*; Cambridge University Press, Cambridge, England, 1995.
15. S. M. Sang, A. N. Lao, H. C. Wang, Z. L. Chen, J. Uzawa, and Y. Fujimoto, *Nat. Prod. Sci.*, 1998, **4**, 268.
16. S. M. Sang, A. N. Lao, H. C. Wang, Z. L. Chen, J. Uzawa, and Y. Fujimoto, *J. Asian. Nat. Prod. Res.*, 1999, **1**, 199.
17. S. M. Sang, A. N. Lao, H. C. Wang, Z. L. Chen, J. Uzawa, and Y. Fujimoto, *J. Asian. Nat. Prod. Res.*, 2000, **2**, 187.
18. S. M. Sang, A. N. Lao, H. C. Wang, Z. L. Chen, J. Uzawa, and Y. Fujimoto, *Chin. Chem. Lett.*, 2000, **11**, 49.
19. S. M. Sang, A. N. Lao, H. C. Wang, Z. L. Chen, J. Uzawa, and Y. Fujimoto, *Tetrahedron Lett.*, 2000, **41**, 9205.
20. S. M. Sang, A. N. Lao, H. C. Wang, Z. L. Chen, J. Uzawa, and Y. Fujimoto, *J. Asian. Nat. Prod. Res.*, in press.
21. S. M. Sang, A. N. Lao, H. C. Wang, Z. L. Chen, J. Uzawa, and Y. Fujimoto, *Phytochemistry*, 1998, **47**, 569.
22. S. M. Sang, A. N. Lao, H. C. Wang, and Z. L. Chen, *Zhongcaoyao*, 2000, **31**, 169.
23. S. M. Sang, A. N. Lao, H. C. Wang, and Z. L. Chen, *Tianran Chanwu Yanjiu Yu Kaifa*, 2000, **12**, 12.
24. Merck Index (Ninth edition), p. 241.
25. N. Fusetani and S. Matsunaga, *Chem. Rev.*, 1993, **93**, 1793.
26. Y. Matsubara, T. Yusa, A. Sawabe, Y. Lizuka, S. Takekuma, and Y. Yoshida, *Agric. Biol. Chem.*, 1991, **55**, 2923.
27. S. Yahara, C. Shigeyama, T. Ura, K. Wakamatsu, T. Yasuhara, and T. Nohara, *Chem. Pharm. Bull.*, 1993, **41**, 703.

28. K. M. Witherup, M. J. Bogusky, P. S. Amderson, H. Ramjit, R. W. Ransom, T. Wood, and M. Sardama, *J. Nat. Prod.*, 1994, **57**, 1619.
29. A. J. J. Van den Berg, S. F. A. J. Horsten, J. J. Kettenes-van den Bosch, B. H. Kroes, C. J. Beukelman, B. R. Leeftang, and R. P. Labadie, *FEBS Lett.*, 1995, **358**, 215.
30. A. J. J. Van den Berg, S. F. A. J. Horsten, J. J. Kettenes-van den Bosch, B. H. Kroes, C. J. Beukelman, B. R. Leeftang, and R. P. Labadie, *Phytochemistry*, 1996, **42**, 129.
31. H. Itokawa, Y. Yun, H. Morita, K. Takeya, and K. Yamada, *Planta Med.*, 1995, **61**, 561.
32. S. M. Sang, A. N. Lao, H. C. Wang, and Z. L. Chen, *Tianran Chanwu Yanjiu Yu Kaifa.*, 1999, **10**, 1.
33. C. Rice-Evans, *Biochem. Soc. Symp.*, 1995, **61**, 103.
34. G. Gao, E. Sofic, and R. L. Prior, *Free Radical Biol. Med.*, 1997, **22**, 749.
35. M. J. Tikkanen, K. Wahala, S. Ojala, V. Vihma, and H. Adlercreutz, *Proc. Natl. Acad. Sci., U.S.A.* 1998, **95**, 3106.
36. R. L. Singhal, Y. A. Yeh, N. Prajda, E. Olah, G. W. Sledge, and G. Weber, *Biochem. Biophys. Res. Commun.*, 1995, **208**, 425.
37. F. V. So, N. Guthrie, A. F. Chambers, M. Moussa, and K. K. Carroll, *Nutr. Cancer*, 1996, **26**, 167.
38. L. G. Menon, R. Kuttan, M. G. Nair, Y. C. Chang, and G. Kuttan, *Nutr. Cancer*, 1998, **30**, 74.
39. M. Messina and S. Barnes, *J. Natl. Cancer Inst.*, 1991, **83**, 541.
40. M. Messina, S. Barnes, and K.D. Setchell, *Lancet*, 1997, **350**, 971.
41. Unpublished data.
42. S. M. Sang, Z. H. Xia, S. L. Mao, A. N. Lao, and Z. L. Chen, *Zhongguo Zhongyao Zazhi*, 2000, **25**, 221.
43. M. T. Rae, G. S. Menzies, A. S. McNeilly, and K. Woad, *Biology of Reproduction*, 1998, **58**, 1394.