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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 amennorrhea and breast infections.[1] Previous studies on the seeds of this species led to the isolation of several triterpenoid saponins,[2-7] seven cyclic peptides[8-11] 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.

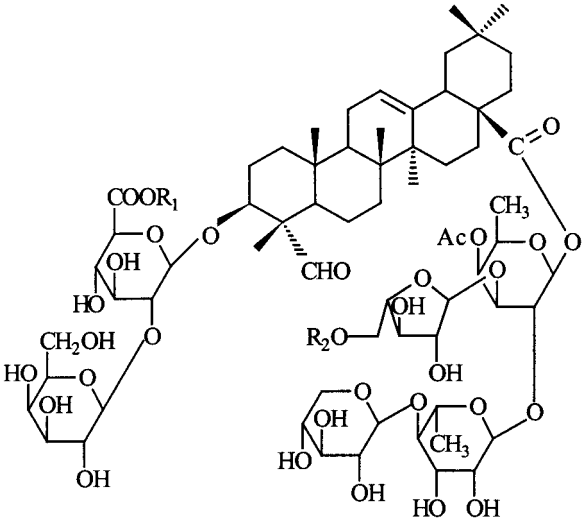
2. Phytochemistry

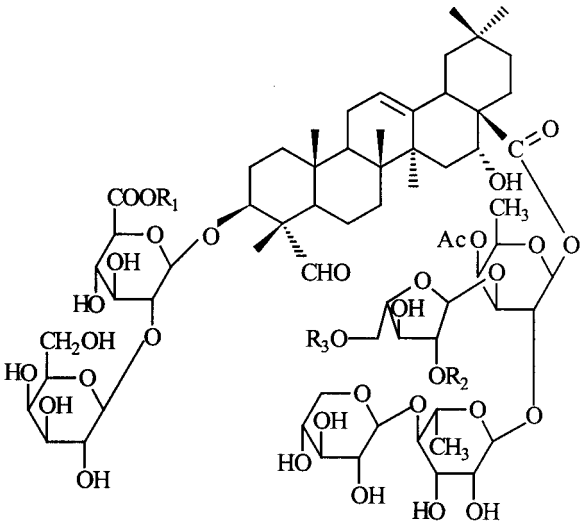
The powdered seeds of *V. segetalis* (50 Kg) were extracted successively with petroleum ether (two times) and 95% EtOH (three times). After evaporation of ethanol *in vacuo,* the residue was suspended in water and then extracted successively with CH_2Cl_2, EtOAc and *n*-BuOH. Forty-five compounds were 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-*seco*gypsogenic acid and oleanolic acid. The aglycons can be further subsituted 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

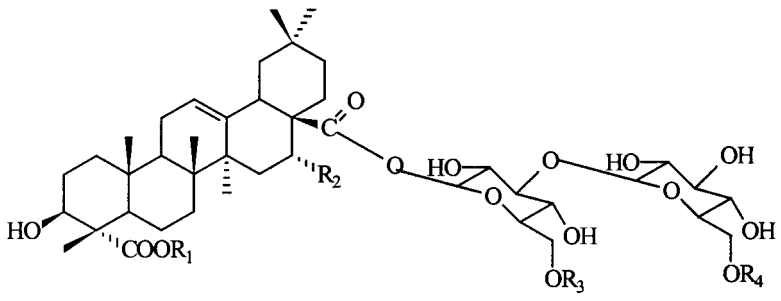
3,4-*seco* derivative of gypsogenic acid type saponins (**21**, **22**)[15-20] and one oleanolic acid type saponin. Fourteen of them (**2**-**11**, **15**, **18**, **19** and **23**) are new compounds.

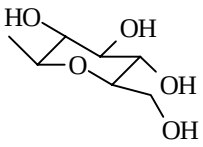


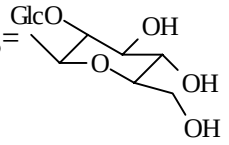


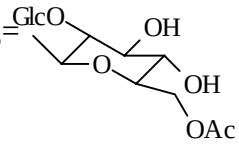
1 : R_1=R_2=H
2*: R_1=H, R_2=Ac
3*: R_1=CH_3, R_2=Ac
4*: R_1=CH_3, R_2=H
5*: R_1=CH_2CH_3, R_2=H
6*: R_1=$CH_2CH_2CH_2CH_3$, R_2=H

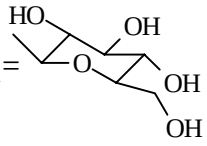
7*: R_1=R_2=H R_3=Ac
8*: R_1=H, R_2=R_3=Ac
9*: R_1=CH_3, R_2=R_3=Ac
10*: R_1=CH_3, R_2=H, R_3=Ac
11*: R_1=$CH_2CH_2CH_2CH_3$, R_2=H, R_3=Ac
12 : R_1=R_2=R_3=H

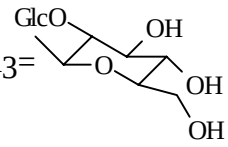


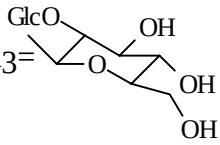
13: $R_1=R_2=R_4=H$, $R_3=$ 

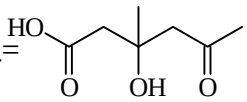
14: $R_1=R_2=R_4=H$, $R_3=$ 

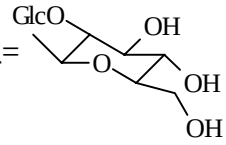
15*: $R_1=R_2=R_4=H$, $R_3=$ 

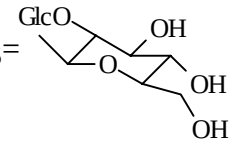
16: $R_1=$ 

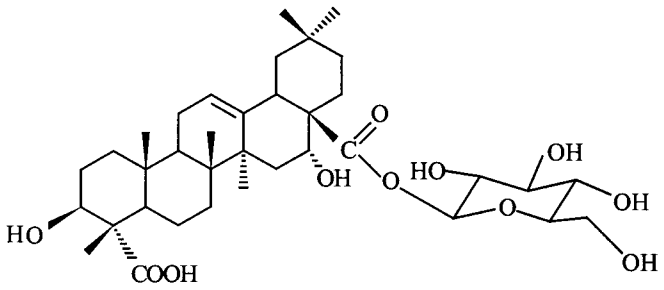
$R_2=R_4=H$, $R_3=$ 

17: $R_1=R_2=H$, $R_3=$ 

$R_4=$ 

18*: $R_1=R_2=R_3=H$, $R_4=$ 

19*: $R_1=R_4=H$, $R_2=OH$, $R_3=$ 



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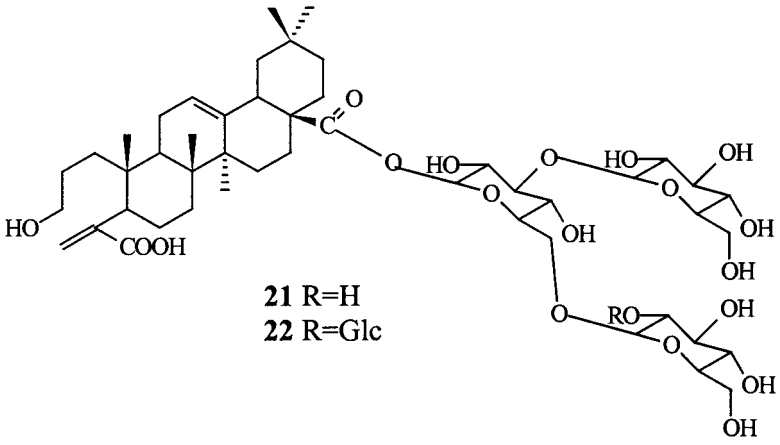
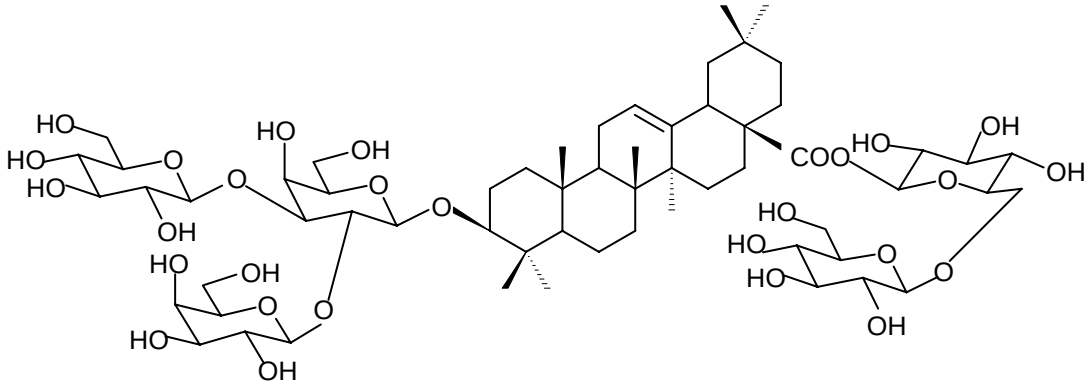


21 R=H
22 R=Glc



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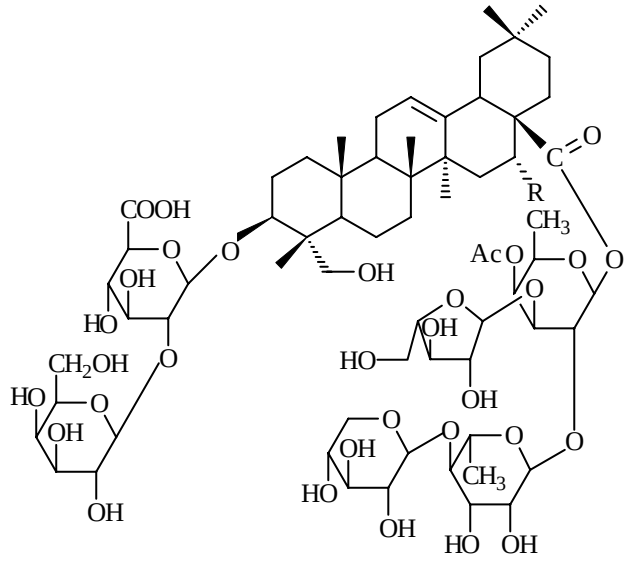
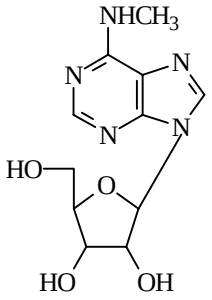
In addition to the triterpenoid saponins that we present here, there are two more compounds (vaccarosides **F** and **H**) reported by Nikaido *et al*. from this seed.[7]



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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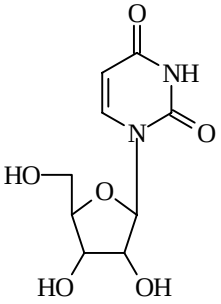

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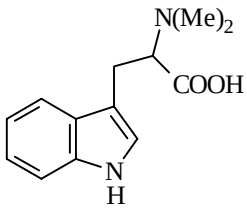

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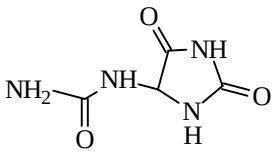

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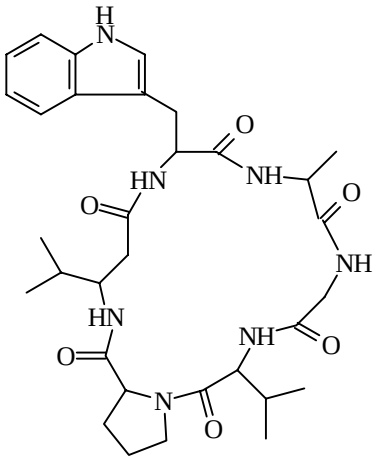
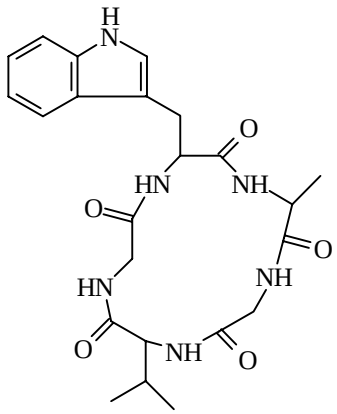
2.3 Cyclic peptides

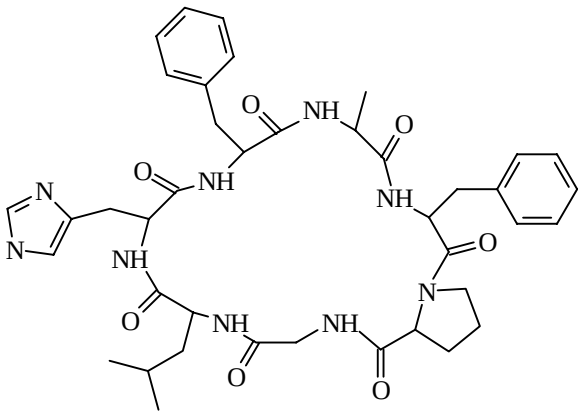
Cyclic peptides are natural products exhibiting a wide variety of biological functions. Large numbers of cyclic peptides with unique structures and various pharmacological activities are reported from marine and microorganisms,[25] whereas only few examples are known from higher plants.[26-30] During 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



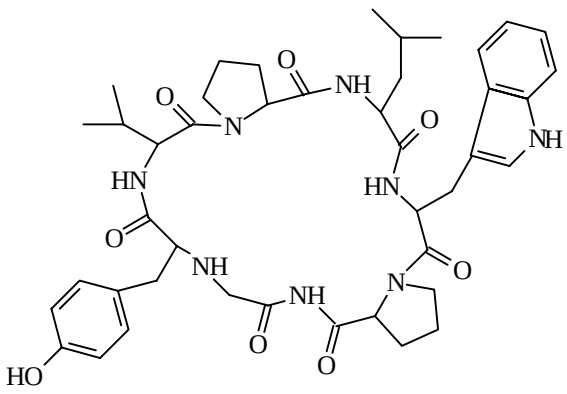
28: Segetalin A



29: Segetalin B

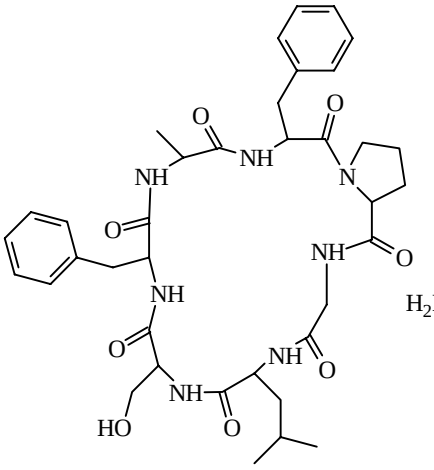


30: Segetalin C

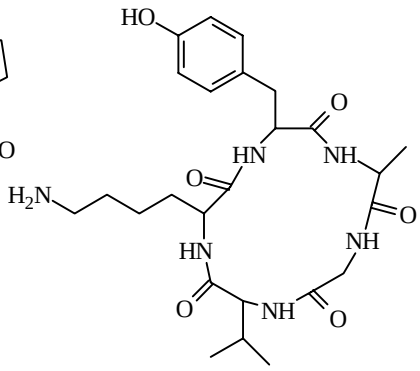


31: Segetalin E

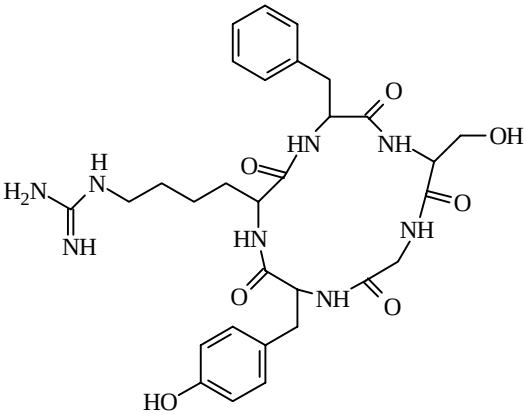
were first reported by Itokawa *et al*., who reported seven cyclic peptides (segetalins **A**-**E**, **G**-**H**) from this species.[8-11] Among these, segetalins **A** (**28**), **B** (**29**), **G** and **H** showed estrogen-like activity assayed by the increment of uterus in ovariectomized rats.[31]



Segetalin **D**



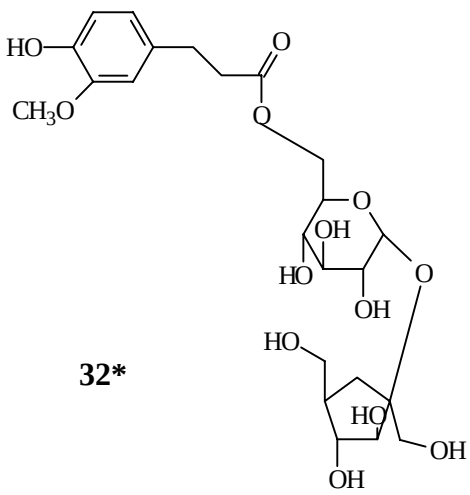
Segetalin **G**



Segetalin **H**

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.



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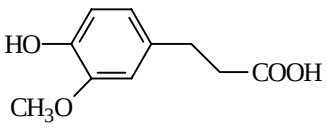

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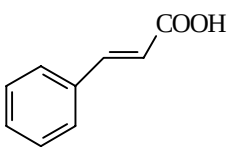

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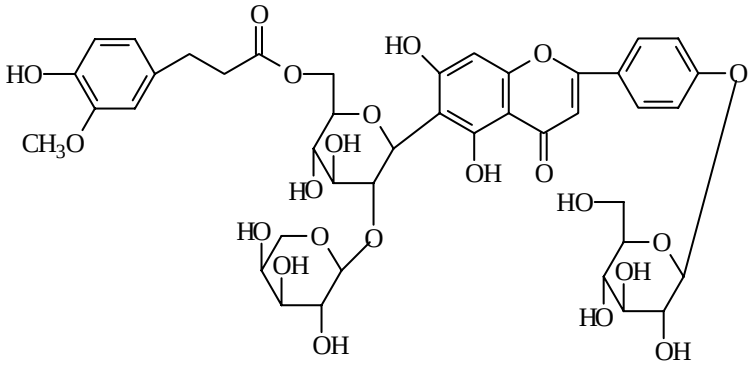
2.5 Flavonoids

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]



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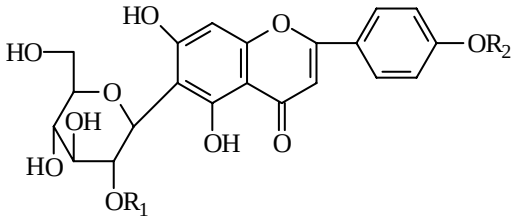
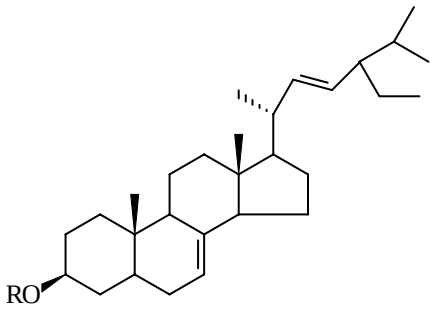


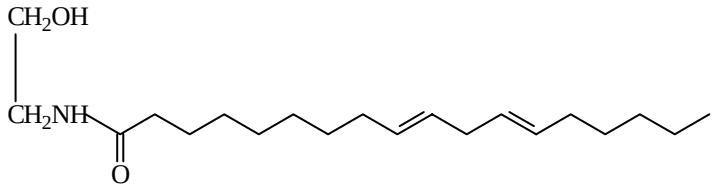
36. R_1=Ara, R_2=H
37. R_1=Glc, R_2=H
38. R_1=Ara, R_2=Glc
39. R1=H, R2=H

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]



40. R=H
41. R=Glc



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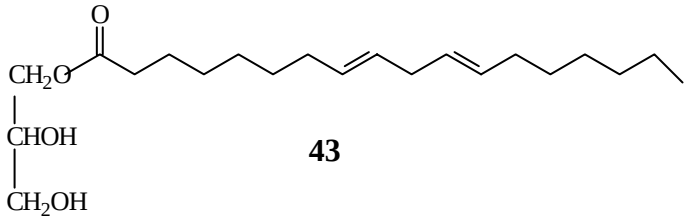

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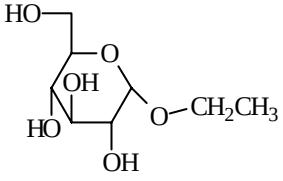

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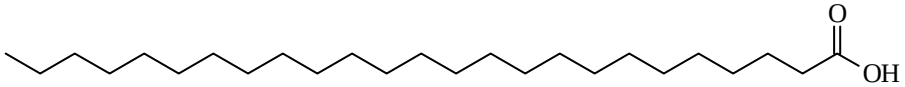

44

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 IC_{50} value of 97.4 μg/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 μg/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 μg/mL. One new flavonoid showed inhibitory effect on the growth of the HL-60 cell line.

ACKNOWLEDGEMENT

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