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BIOLOGICALLY ACTIVE SUBSTANCES FROM MUSHROOMS IN YUNNAN, CHINA

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Abstract – As a part of our search for naturally occurring bioactive metablites of mushrooms, we investigated the chemical constituents of eight Basidiomycetes and Ascomycetes fungi (*Albatrellus confluens*, *Russula lepida*, *Thelephora ganbajun*, *Russula cyanoxantha*, *Polyporus ellisii*, *Tuber indicum*, *Engleromyces gotzii*, *Tremella aurantilba*, *Laetiporus sulphureus* and *Tylopilus plumbeoviolaceus*), and isolated a number of novel terpenoids, phenolics and nitrogen-containing compounds. The isolation, structural elucidation and biologically activity of the new compounds are discussed.

1. Introduction

Yunnan Province, southwest of China, is one of the areas with the richest and diverse bioresources in the world based on its unique geoenviroment, diverse geomorphology and three-dimensioal differentiation of climate. About 1500 species of mushrooms are now known in Yunnan. Edible and inedible

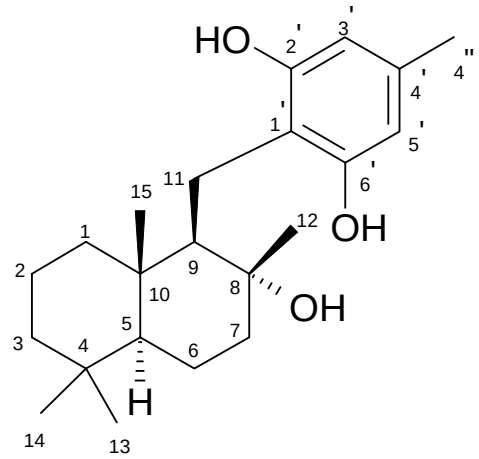
mushrooms are about 600 and 900 species, including 10 poionous species. Mushrooms in bioresources belong to the very productive biologically sources which produce a large and diverse variety of secondary metabolites. We have been interested in the biologically active substances present in untapped and diverse source of mushrooms from Yunnan.

Recently several dozen new natural products and bioactive compounds were found in selected mushrooms on the basis of using our knowledge on the collection of fruiting bodies, strain preservation, fermentation, biologically screening and chemical investigation of mushrooms. The isolation, structural elucidation and biologically activity of the novel terpenoids, phenolics and nitrogen-containing compounds from Basidiomycetes and Ascomycetes fungi (*Albatrellus confluens*, *Russula lepida*, *Thelephora ganbajun*, *Russula cyanoxantha*, *Poluporus ellisii*, *Tuber indicum*, *Engleromyces gotzii*, *Tremella aurantilba*, *Laetiporus sulphureus* and *Tylopilus plumbeoviolaceus*) will be discussed in this review.

2. Activities of Albaconol, a Novel Prenylated Resorcinol from the Basidiomycete *Albatrellus confluens* on the Human Vanilloid Receptor 1 (VR1)[1]

Rapid progress is being made in the area of therapeutic vanilloids in recent years, including the discovery of compounds that are recognized by vanilloid receptors but do not have the homovanillyl moiety. These achievements should provide even more tools for further understanding pain at the molecular level and for the design of new painkillers.[2] The compound scutigeral, a non-pungent triprenyl phenol from *Albatrellus ovinus*, was reported that it stimulates rat dorsal root ganglion neurons via interaction at vanilloid receptors and may act as orally active, nonpainful painkiller targeting vanilloid receptors.[3] As part of a search for naturally occurring bioactive metabolites of the higher fungi in Yunnan Province and for analogues of scutigeral type compounds, the chemical composition of the inedible mushroom *Albatrellus confluens* collected inYunnan was investigated. It was found that the fruiting bodies of *A. confluens* contained high concentrations of albaconol (**1**), a new prenylated diphenol. The effects of albaconol on vanilloid receptors were studied electrophysiologically on rat ganglion neutrons as well as on recombinant cell lines expressing rat VR1 receptor. The results of these investigations suggested that albaconol (**1**) acts as VR1 antagonists (IC_{50} 5 microM).

3. Antifungal and Farnesyl Transferase Inhibitory Activities of New Terpenoids from the Basidiomycete *Russula lepida*[4, 5]



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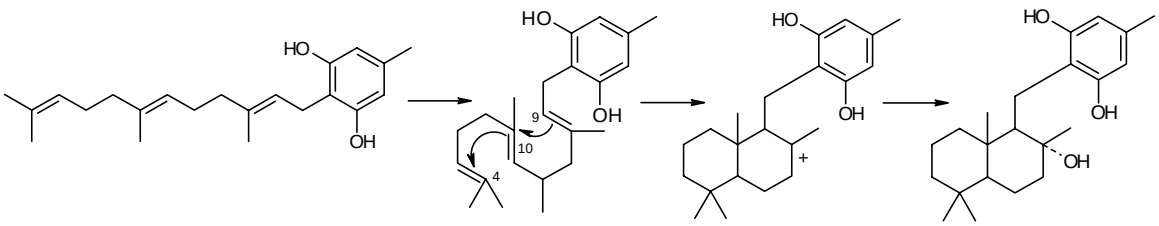
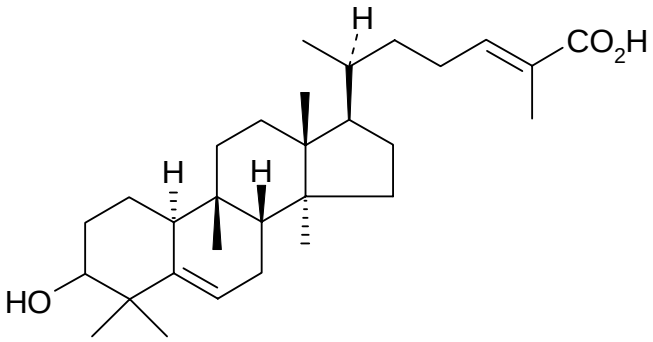


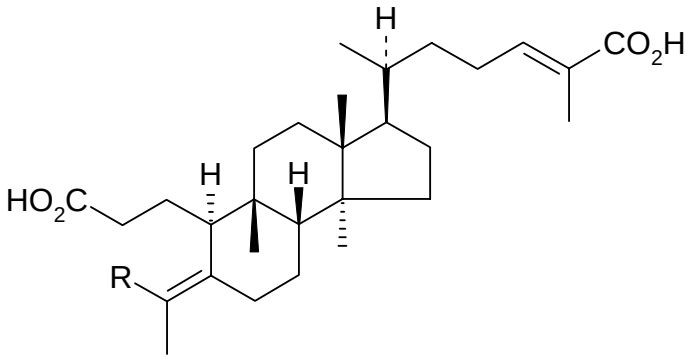
Proposed Biosynthetic Route to Albaconol (**1**)

The Russulaceae family is one of the largest in the subdivision Basidiomycotina in Witthaker's Kingdom of Fungi and comprises hundreds of species.[6] While secondary metabolites occurring in the fruiting bodies of European *Lactarius* species have been well investigated, the *Russula* mushrooms have received less attention, notwithstanding the larger number of existing species.[7] *Russula lepida* Fr. has been used as a food and medicinal agent in China. The extract of its fruiting bodies showed anti-tumor activity.[8] Four new triterpenoids and three new aristolane sesquiterpenoids, namely (24*E*)-3β-hydroxycucurbita-5, 24-dien-26-oic acid (**2**), (24*E*)-3,4-*seco*-cucurbita-4,24-diene-3,26-dioic acid (**3**), (24*E*)-3,4-*seco*-cucurbi-ta-4,24-diene-3,26,29-trioic acid (**4**), rulepidadiol (**5**), rulepidatriol (**6**), rulepidol (**7**) and lepidolide (**8)** have been isolated from the fruiting bodies of Basidiomycetes *Russula lepida*. Their structures were established by spectral methods. Compounds (**3, 4** and **8)** are the first example of naturally occurring *seco*-ring-A cucurbitane triterpenoids. Compounds (**5**, **6** and **7)** belonging to the aristolane type

sesquiterpenoid are of a type rather rare in the fungal species. Vidari *et al*. reported the isolation of several other aristolane-type sesquiterpenes from the same mushroom.[7] Our bioassay indicated that compound (**2)** showed antifungal activity (YNG-CA, IC_{50} 2.9 μg/mL; YNG-CG, IC_{50} 2.3 μg/mL) and compound (**3)** showed farnesyl transferase inhibitory activity (IC_{50} 24 μg/mL).

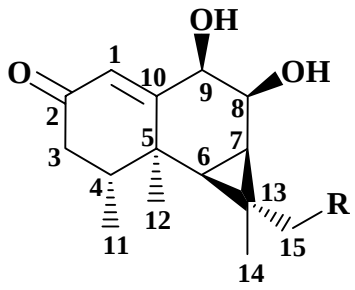


(24*E*)-3β-hydroxycucurbita-5, 24-diene-26-oic acid (**2**)

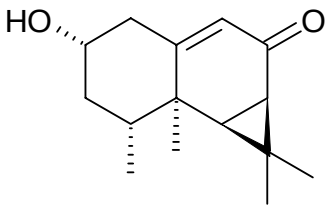


(24*E*)-3,4-*seco*-cucurbita-4, 24-diene-3, 26-dioic acid (**3**) R=CH_3

(24*E*)-3,4-*seco*-cucurbita-4, 24-diene-3, 26, 29-trioic acid (**4**) R=CO_2H



R=H (**5)**; R=OH (**6**)



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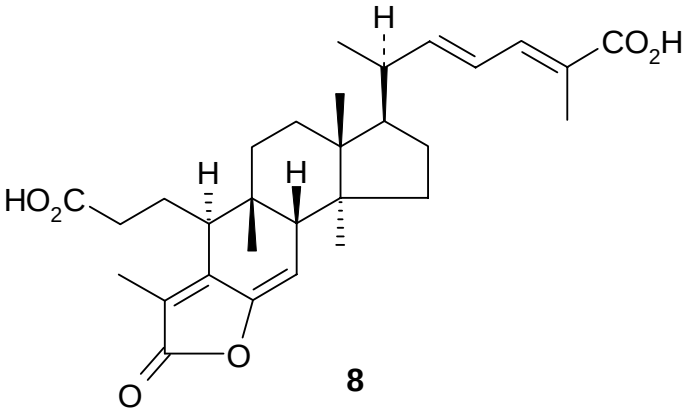

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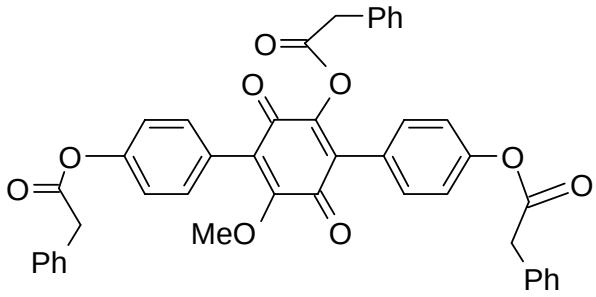
4. Seven Unusual Polyphenylacetoxylated *p*-Terphenyl Derivatives from Fruiting Bodies of the Basidiomycete *Thelephora ganbajun*[9, 10]

Thelephora ganbajun (Basidiomycetes), locally known as 'Gan-Ba-Jun', is mushroom which grows in symbiosis with pine trees found in Yunnan Province, China. It is one of the most favorite edible mushrooms in Yunnan and has a gastronomic interest due to its unique flavor and taste. Despite its commercial value and special flavor, *T. ganbajun* and the other species of same genus have been poorly studied in respect to their contents of secondary metabolites. In the course of our search for naturally occurring bioactive metabolites of higher fungi in Yunnan Province, the chemical constituents of *T. ganbajun* were investigated. Surprisingly, a series of new polyphenylacetoxylated *p*-terphenyl derivatives (**9**-**15**) were isolated from this fungus. Phenylacetoxylated *p*-terphenyls have not been reported yet in the literature.

In recent years, it has been reported that several *p*-terphenyl compounds exhibit considerable bioactivities, such as active toward HeLa cells,[11] potent IgE antibody suppressant,[12] antiinsect and antibacterial,[13] specific 5-lipoxygenase inhibitory.[14] Because of their promising biological activities, they have created much increasing research interest.

5. Bioactive Sphingolipids from Basidiomycetes *Russula cyanoxantha*, *Polyporus ellisii* and Chinese Truffles *Tuber indicum*



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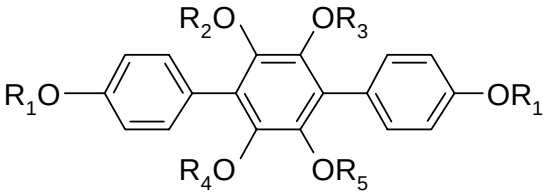
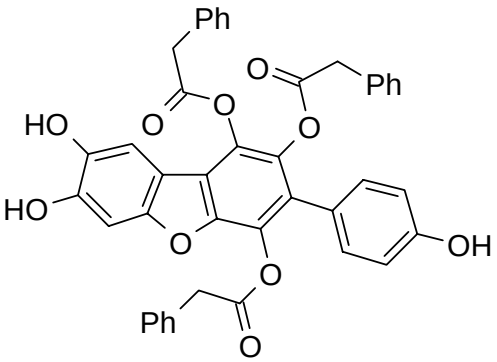


11 $R_1=COCH_2Ph$, $R_2=R_3=R_4=R_5=H$
12 $R_1=R_3=R_5=H$, $R_2=R_4=COCH_2Ph$
13 $R_1=R_3=R_4=H$, $R_2=R_5=COCH_2Ph$



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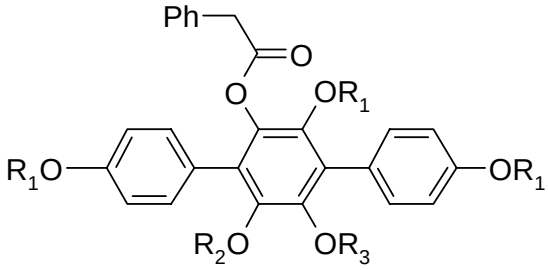
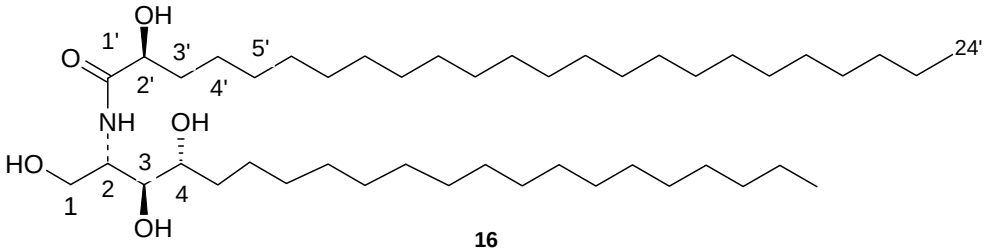


14 $R_1=R_3=H$, $R_2=OCH_3$
15 $R_1=R_2=H$, $R_3=OCH_3$

Sphingolipids are important building blocks of the plasma membrane of eukaryotic cells. In recent years, however, a great deal of attention has been devoted to studies of the biological processes regulated by sphingolipids, and evidence of newly discovered roles of these compounds in cell functions is continually being demonstrated. Their function is to anchor lipid-bound carbohydrates to cell surfaces and to creat an epidemal water permeability barrier, as well as to participate in antigen-antibody reactions and transmission of biological information.[15,16] Some are also anti-uncerogenic, ionophoretic, antihepatotoxic, antitumor, immunostimulatory or stimulatory to axon growth.

We investigated the chemical constituents of the mushrooms, *Russula cyanoxantha*, *Polyporus ellisii* . New ceramide (**16**) and new glycosphingolipid (**17**) containing an unusual sphingoid base from the basidiomycetes above mentioned have been isolated, respectively.[17,18]

Truffles, also known as the “black diamonds”, are underground mushrooms which grow in symbiosis with certain trees. They are thought to be a “miracle of nature” and have been, since ancient times, the ultimate in gastronomy. There are more than a hundred different kinds of truffles, but only a few have a gastronomic interest, some are not edible. The Chinese truffle *Tuber indicum*, a generally hypogeous fungus belonging to the family Tuberaceae, is distributed in the provinces of Yunnan and Sichuan of China. This truffle looks a lot like the black winter truffle *Tuber melanosporum* in Europe. Its skin is reddish before becoming brownblack. The flesh is white veins and has an elasticized consistency. Since its significant commercial value the chemical composition of *T. indicum* was investigated. From the fruiting bodies of *T. indicum* four ceramides (**18**-**21**) except the reported new sterol were isolated.[19] Preliminary study indicated that compound (**20**) showed only weak phospholipase A_2 (PLA_2) inhibitory activity.



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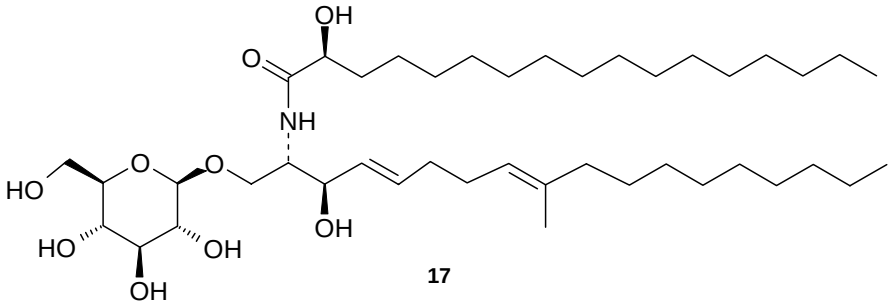

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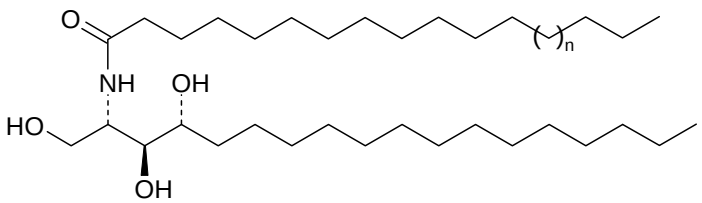
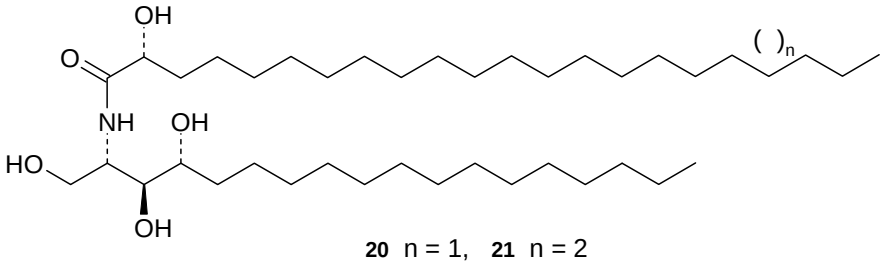


18 n = 7, **19** n = 4

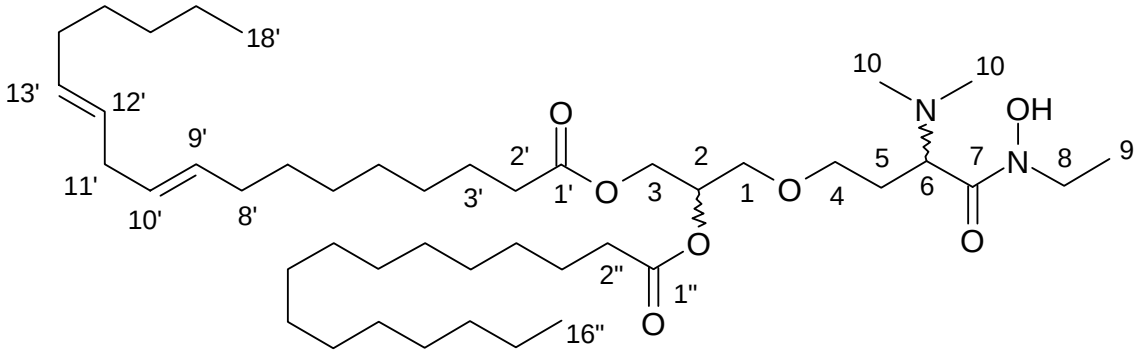


20 n = 1, **21** n = 2

6. Other Interesting Compounds from Mushrooms *Engleromyces gotzii*, *Tremella aurantilba* and *Laetiporus sulphureus*

The fungus *Engleromyces gotzii* belongs to Hypocreaceae and grows on the bamboo of high mountains. It has been used as a folk remedy against infection and cancer diseases in the area around Tibet of China including Yunnan and Sichuan Provinces. Little attention has been paid to the chemical constituents of this inedible fungus. One novel compound named neoengleromycin (**22**) was isolated from the fungus *E. gotzii*, together with two known cytochalasins: cytochalasin D (**23**) and 19, 20-epoxycytochalasin D (**24**). Another new compound named tremellin (**25**) was also isolated from the basidiomycete *Tremella aurantilba*, the configuration was determined by X-Ray crystallography.

Appleton reported in 1988 that *Laetiporus sulphureus* caused visual hallucinations and ataxia in a child.[20] Two compounds (**26**, **27**) were isolated from the fruiting bodies of mushroom *Laetiporus sulphureus*. Animal test *in vivo* (monkey, intramuscular injection **26**, **27**, 3.8 mg/kg) showed the dopamine D_2 receptor agonist like activity. Selective dopamine D_2 receptor agonist was used in treating Parkinson's disease in clinic.



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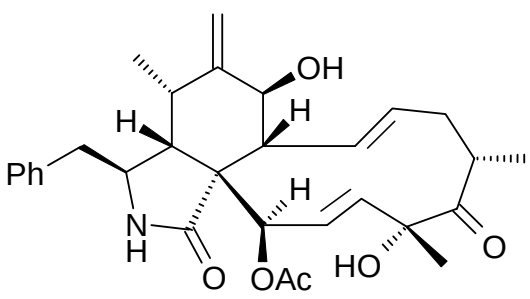

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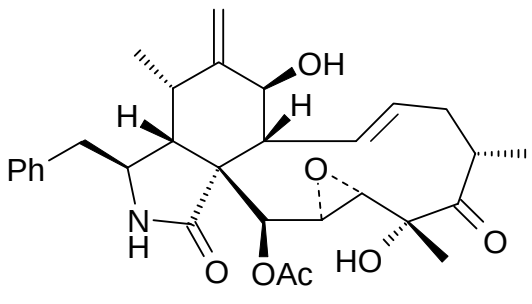

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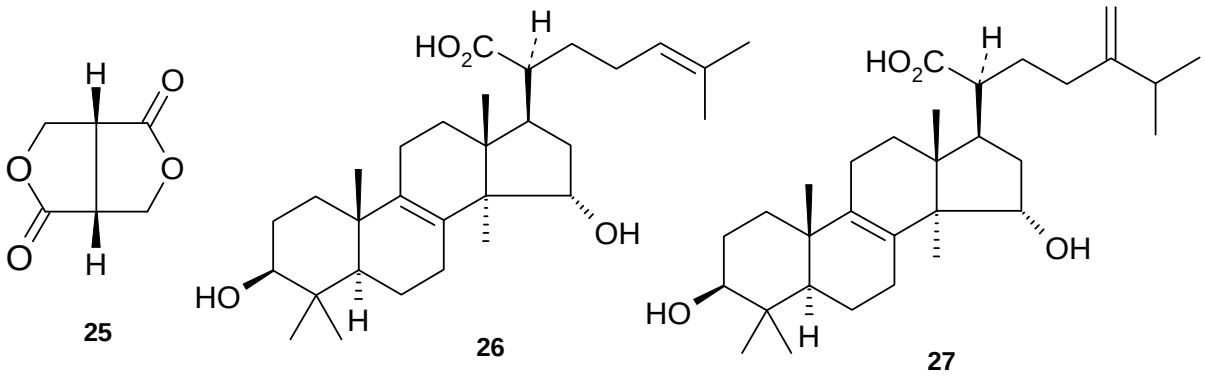
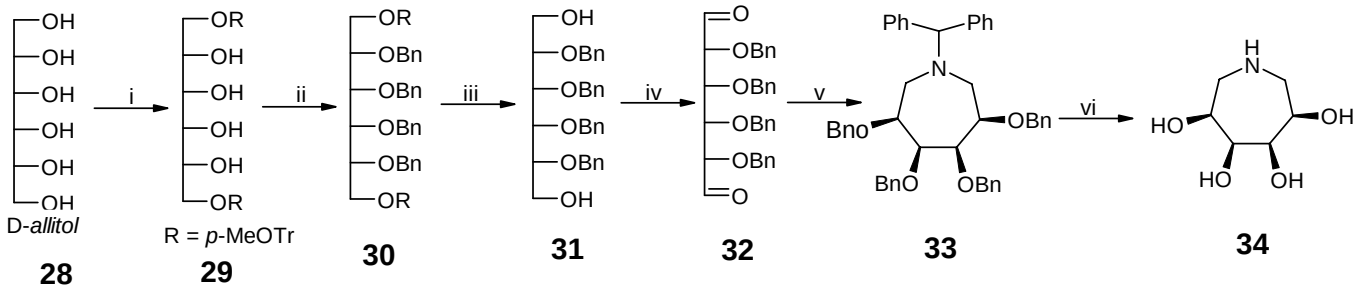


7. Synthesis of (3*S*, 4*R*, 5*S*, 6*R*)-Tetrahydroxyazepane from D-Allitol of Mushrooms

Inhibitiors of some of glycosidase and glycosyltransferases have been implicated in the treatment of diabetes and other metabolic disorders as well as blocking microbial infections and metastasis. In the past decade, azasugars have attracted attentions of many organic and medicinal chemists because of their potential values as therapeutic agents for the treatment of cancer, diabetes and AIDS. Much work has been devoted to the preparation and subsequent evaluation of five-and six-membered azasugars, however only a few reports have appeared on the synthesis of the seven-membered analogues despite their inhibitory potential. Synthesis of the polyhydroxylated azepane (3*S*, 4*R*, 5*S*, 6*R*)- tetrahydroxyazepane (**1**) from a naturally occurring *D*-allitol was described. Key step includes the double reductive amination of 1,6-diformyl derivative. Cleavage of all the protecting groups of **7** was accomplished in one step under hydrogenation condition. This is the first report on synthesis of tetrahydroxyazepane from *D*-allitol.



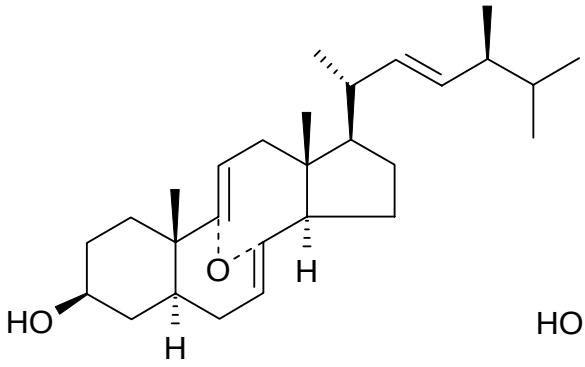
Scheme **1.** ***Reagents and conditions*:**

(i), *p*-methoxytrityl chloride, DMAP, pyridine, rt, 90%; (ii), BnBr, NaH, THF, 0 to rt ℃, 90%; (iii), H_2SO_4, CH_3OH/CH_2Cl_2, 0 ℃; (iv), DMSO, $(COCl)_2$, $(C_2H_5)_3N$, CH_2Cl_2, -60 ℃ to rt; (v), $NaCNBH_3$, Ph_2CHNH_2, AcOH, 3Å MS, CH_3OH, -78 ℃ to rt; (vi), $Pd(OH)_2/C$, H_2, CH_3OH.

8. Two Novel Secoergosterols from Inedible Mushroom *Tylopilus plumbeoviolaceus*

Tylopilus plumbeoviolaceus is inedible, bitter fungus belonging to the family Strobilomycetaceae, it is

distributed widely in the central aera of Yunnan Province. Two new compounds named tylopiol A (**35**) and tylopiol B (**36**) were isolated from the fruiting bodies. The structures of these two compounds are based on the ergostane skeleton, in which the bond between C-8 and C-9 is cleaved to form an enol ether oriented in the α position. Although this type of modified skeleton is very rare, a similar ergostane compound, jereisterol A, has been isolated from a marine sponge. The structures and stereochemistry of tylopiol A and tylopiol B were confirmed by X-Ray crystallography.[21]



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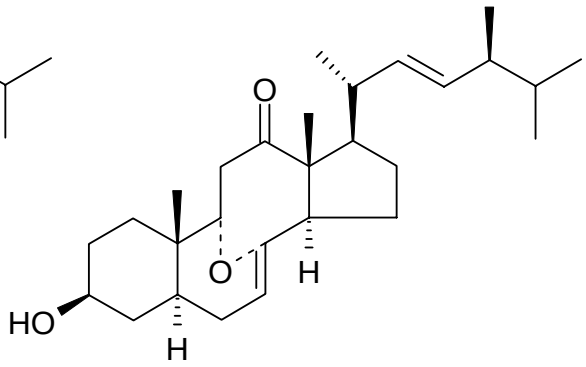

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