# A CONTRIBUTION TO ATTENUATION OF HEALTH PROBLEMS IN BOLIVIA: BIOACTIVE NATURAL COMPOUNDS FROM NATIVE PLANTS REPORTED IN TRADITIONAL MEDICINE

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**Key Words:** Spectral Analysis, Pharmacognosy, Traditional Medicine, Natural Substances, Antiparasitic, Antibacterial, Antifungal, Bolivia.

## RESUMEN

Un estudio fitoquímico bioguiado por ensayos biológicos antiparasitarios (*Plasmodium falciparum, P. berghei, Leishmania braziliensis, L. amazonensis, L. donovani y Trypanosoma cruzi*), antibacterianos (*Shigella flexneri, Staphylococcus aureus* y otras bacterias) y antifúngicos (*Neurospora crassa* y *Candida albicans*) de seis plantas bolivianas fue realizado. Las especies fueron seleccionadas de farmacopeas de tres etnias Bolivianas; de la etnia Chácobo: *Amburana cearensis* (Fabaceae) y *Qualea paraensis* (Vochysiaceae); de la etnia Raqaypampeños: *Dunalia brachyacantha* (Solanaceae) y *Notholaena nivea var. flava* (Pteridaceae); y de la etnia Tacana: *Cavanillesia aff. hylogeiton* (Bombacaceae). La especie *Senecio smithioides* (Asteraceae) reportada en la farmacopea Kallawaya fue igualmente estudiada. Diez y ocho compuestos potencialmente activos (entre ellos cinco de estructura nueva), fueron identificados a través de análisis espectroscópicos. Los resultados más significativos corresponden a un sesquiterpeno antipalúdico, dos withanólidos trypanocidas y leishmanicidas, y dos flavanonas antifúngicas.

## ABSTRACT

A phytochemical study monitored by antiparasite (*Plasmodium falciparum, Leishmania braziliensis, L. amazonensis, L. donovani* and *Trypanosoma cruzi*), antibacterial (*Shigella flexneri, Staphylococcus aureus* and other bacteria) and antifungal (*Neurospora crassa* and *Candida albicans*) assays of six bolivian plants has been achieved. The plants were collected according to ethnobotanic criteria from three bolivian ethnic groups; from the Chacobo ethnic group; *Amburana cearensis* (Fabaceae) and *Qualea paraensis* (Vochysiaceae), from the Raqaypampeños ethnic group: *Dunalia brachyacantha* (Solanaceae) and *Notholaena nivea* var. *flava* (Pteridaceae) and from the Tacanas ethnic group: *Cavanillesia aff. hylogeiton* (Bombacaceae). The species *Senecio smithioides* (Asteraceae) reported in the Kallawaya Pharmacopoeia has also been studied. Eighteen pure compounds (comprising five new structures) potentially actives were identified by spectroscopic methods. The identified active principles, concern one antimalarial sesquiterpene, two potent leishmanicidal and trypanocidal withanolides and two antifungal flavanones.

## INTRODUCTION

An important percentage of the Bolivian population exhibits very low public health levels, settling the country among the more affected in Latin America. Rural people particularly in the tropics suffer of parasite diseases. The diarrhea specially in children is connected to low immunology caused by malnutrition, and the respiratory as well as fungal diseases are both common in population of scarce economic, social and cultural resources. A survey of remedies based on plants reported in Bolivian traditional native pharmacopoeia, constitutes a good field to start a quest of anti-parasite, and anti-infectious active principles. Plants reported by three ethnic groups, the Tacanas, the Chacobos and the Raqaypampeños have been selected for a exhausting-intended-search of active molecules. The Tacanas and Chacobos are settled in the northern-eastern tropical regions of the country. The Raqaypampeños ethnic group is situated in the inter Andean valleys or transition geographic places between the tropics and the high lands.

## RESULTS AND DISCUSSION

Since 1993, a Bolivian French team of chemists and biologists is devoted to the valorization of vegetal species used in traditional medicine by Bolivian ethnic groups. Plants have been chosen taking into account parasitic endemics like malaria, leishmaniasis and Chagas' disease as well as infectious affections in Bolivia. The appropriate approach for such an evaluation consists of *in vitro* and *in vivo* bio-assays applied to vegetal extracts and subsequent tandem bio-guided separation and isolation of compounds. The tests include antibacterial and antifungal assays as well. Isolated secondary metabolites come from six medicinal species from Bolivia: *Senecio smithioides* Cabrera (Asteraceae), *Amburana cearensis* A. C. Smith (Fabaceae), *Qualea paraensis* Ducke (Vochysiaceae), *Dunalia brachyacantha* Miers (Solanaceae), *Notholaena nivea* var. *flava* Hook (Pteridaceae) and *Cavanillesia aff. hylogeiton* Ulbr. (Bombacaceae). Plants were provided by the ethnic groups Tacanas, Chacobo and Raqaypampeños. From a chemical and biological stand point we found a supplementary interest in studying these species cause of a lack of, or of a little of previous reported studies. The discovery of new active principles should justify our work that fall into the "ethnopharmacologic" category[1]. In Bolivia, as well as in the tropical world in general, parasitical problems, namely malaria, leishmaniosis and Chagas' disease, reach alarming indexes as it can be seen in epidemiological published data[2,3,4,5]. The six selected plants were previously tested throughout a pharmacological screening of their hydroethanolic extracts over 300 vegetal species harvested principally in tropical regions of Bolivia, (see Table I). The employed methodology in the search of active principles reposes on the extraction and isolation by the bias of protocols oriented by the results obtained from the pharmacological screening. Isolated products are then submitted to structural elucidation. Two kind of extraction procedures were applied. The most employed consists of a classic exhausting extraction of the plant's organ previously dried and powdered, using increasing-extracting-power solvents. The second procedure applies a first hydroethanol extraction to obtain an hydroethanolic extract. After elimination of alcohol this is mixed with water to be extracted liquid-to-liquid with dichloromethane (DM). The corresponding organic layer is concentrated at reduced pressure. The dried residue (DM extract) is dissolved in petroleum ether (PE) and extracted liquid-to-liquid with a hydromethanolic mixture. Various chromatography techniques (VLC, LC, TLC, PTLC) were used to afford our nineteen potential active principles. Structural elucidation was carried out mainly by NMR analyses and correlated to MS analyses. Planar structures of genines were deduced from HMBC, HMQC, XHCORR and COLOC experiments results. For stereochemical attributions we employed the NOESY and ROESY experiments. Sugar moieties when pertinent, were deduced structurally throughout the use of COSY, HOHAHA, ROESY and HMBC. To derivate compounds chemically was applicable in necessary cases to clarify structural definitions based on NMR analyses. The principal results are listed below (Table II). The species *Senecio smithioides* was studied for the first time. The isolation of the responsible of the extracts initially reported antimalarial activity was achieved at once. The structural characterization of this antimalarial principle indicated that it possesses a twice previously reported structure. It corresponds to furanoeremophil-1(10)-en-9-one[6] (**1**) also known as 9-oxoeuryopsine. This compound has been found in *Euryops hebecarpus* and *S. serratifolius*.[7] The activity measured and repeated in *in vitro* antimalarial assays was referred as an $IC_{50}$ = 1.2 µg/ml over chloroquine sensitive strain (CSS) of *Plasmodium falciparum*. The compound is the major component of the PE extract, itself manifesting an activity corresponding to an $IC_{50}$ close to 1µg/ml. This antimalarial activity index let attribute the responsibility of the antimalarial potentiality contained in the plant. However a lacking cytotoxic study to establish its therapeutic selectivity for compound **1** is recommended. Coumarin[8] (**2**) or 2H-1-benzopyran-2-one present as the major compound of *Amburana cearensis*, seems to be the antimalarial active principle against *Plasmodium falciparum* ($IC_{50}$ 9 µg/ml, CSS). This constitutes the largely major component in the PE and DM extracts practiced to the stem bark. The presence of coumarin in grains of species *Amburana* has been already published, been this the first report on its antimalarial activity. The phenolics amburoside A (**3**) an B (**4**), are novel structures. Only compound **3** manifested a considerable antiplasmodial activity next to that of coumarin, corresponding to an $IC_{50}$ 9 µg/ml, CSS. Compound **3** can be considered as the responsible of the DM extract activity. Antibacterial activity of coumarin (**2**) remains interesting with an inhibition of the culture growing of *Escherichia coli* and *Shigella flexneri* at125 µg/ml. Due to a low cytotoxicity of this product, we could envision the realization of tests of it as antibacterial in animals in an appropriate model (not exposed here). The activity indexes obtained in laboratory, concord with ethnopharmacological information transmitted by inhabitants of the Chacobo ethnic group. This constitutes the first approach to medicinal capabilities of coumarin (**2**) as an antiplasmodial. Also this was a first approach to evaluate its leishmanicidal and trypanocidal eventual properties. Among the extracts obtained from *Qualea*

*paraensis*, the two more active extracts against *Plasmodium falciparum* were the PE and MeOH extracts. The PE extract was a high complexity composition mixture that after chromatography afforded three minor pure compounds and a non resolved mixture with the closest to the native extract antiplasmodial activity ($IC_{50}$ 4.2 µg/ml, CSS). A medium antiplasmodial activity was manifested by compound **5** (3-β-acetoxyurs-12-en-11-one, $IC_{50}$ 9 µg/ml) and **7** (sitosterol, 6.0 µg/ml). No activity was found for **6** (glut-5-en-3β-yl acetate[9]). The possibility of a synergic role for compounds **5** an **7** in the native extract could give an explanation of a major activity index of this, related to individual and isolated activities of pure compounds. The 3-β-acetoxyurs-12-en-11-one[10,11,12] is reported here for the first time as a natural substance being hence a new natural product. This product has been reported in the literature as an hemysynthetic product of the corresponding 3-OH natural precursor : 3-β-hydroxyurs-12-en-11-one, itself previously isolated from *Canarium zeylanicum* and *Ilex goshiensis*. The bio guided separation of fractions obtained form *Dunalia brachyacantha* conducted to two active molecules: withanolides **8** and **9** [13] both with good trypanocidal and leishmanicidal activity. The total lysis of parasites *Trypanosoma cruzi*, is achieved at concentrations of only 25 and 10 µg/ml and the disappearing of 50% of epimastigote and promastigote forms of *Leishmania braziliensis* at the minimal dose of 1 µg/ml. A remarkable antibacterial activity at 0.0625 mg/ml against *Bacillus subtilis* and at 0.125 mg/ml for *Staphylococcus aureus* was discovered for both products. The well reputed anti tumor activity for withanolides should impose a cytotoxicity study for **8** and **9** (not exposed here). Then **8** and **9** appear as the first leishmanicidal and trypanocidal withanolides reported to present. As activities of **8** and **9** against epimastigotes and promastigotes are very close to that manifested by the native extract, we consider them as the active principles contained in the plant. The recollection of the vegetal species *Notholaena nivea* var. *flava* in the Raqaypampeños territory was guided by local pharmacological reports suggesting a potential antibacterial activity. The primary antibacterial and antifungal pharmacological screening results oriented us to the isolation of antifungal active principles. Chromatography treatment of the active antifungal extract conducted the research to the isolation of four flavonoids, two of them antifungals. Flavanones pinocembrin[14] (**12**) and sakuranetin[15] (**14**) manifested a good activity against fungus namely *Neurospora crassa* with 13 and 20 mm of diameter inhibition of the fungal growing in a Petri swell at 0.18 µg/ml. Two other known compounds were isolated and identified as pinostrobine[16] (**13**) and 2',6'-dihydro-4'-méthoxy-dihydrochalcone[16] (**15**). This a first bio guided study of the species that afforded two antifungal principles: pinocembrine and sakuranetin. The initial antimalarial activity (CSS and CRS) of 100% of inhibition of the parasitaemia demonstrated by the DM extract was reencountered in the first fractioning of extracts of the species *Cavanillesia aff. Hylogeiton*.

From such fractions we isolated lupeol (**16**), glucosyl sitosterol (**19**), sitosterol (**17**) and sitostenone (**18**). These compounds showed no biological activity. Repetition of bio assays over the first active fractions showed a loss of activity due certainly to an instability of active principles.

## PERSPECTIVES

Compounds **8** and **9** are very interesting because of their trypanocidal activity, however biological studies must be continued. The antiplasmodial activity of extracts, fractions and pure products should be evaluated. It could be interesting to deep in the researches on *N. nivea* var. *flava* in order to find more active antifungal principles. Thanks to the proved antiparasite and antifungal activity of these species, they are candidates for complementary studies for developing phyto medicines in a sustainable development project. This kind of projects are sponsored by the Bolivian government and they are addressed to minor ethnic groups which have in this manner the opportunity to exploit their medicinal species. An exploitation plan includes primarily a pilot plant for extraction and preparation of simple galenic forms, for example crèmes. It is possible to imagen a sustainable development project for *D. brachyacantha* to produce a phyto medicine against cutaneous forms of lesihmaniasis (*L. amazonensis*). The same can be envisioned for an antigungal produced from extracts of *N. nivea* var. *flava*. We should contemplate for these plants all pertinent previous controlled clinic assays according to official regulations, in order to planify a development project.

## CONCLUSIONS

This pluridisciplinary research regrouped ethnobotanics, chemists and biochemists and conducted to the identification of five antiplasmodial, two trypanocidal and leishmanicidal, three antibacterial and two antifungal principles. We discovered two vegetal species that could represent, after supplementay studies, two easely workly up sources for simple prepared medicines against disesases touching an important percentage of

Bolivian disfavored population, namely: *Dunalia brachyacantha* and *Notholaena nivea* var. *flava.*

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Table I. Results of biological screening over the plants' extracts

| Plant | Extract* | Activity | Inhibition** |
|---|---|---|---|
| *Amburana cearensis*<br>Stem bark<br>Chacobo | PE<br>DM<br>EA | antimalarial<br>antimalarial<br>antimalarial | 70% at 100 µg/ml<br>47% at 10 µg/ml<br>96% at 100 µg/ml<br>63% at 10 µg/ml<br>97% at 100 µg/ml |
| *Qualea paraensis*<br>Stem bark<br>Chacobo | PE<br>MeOH | antimalarial<br>antimalarial | $CI_{50}$ < 4,2 µg/ml CSS<br>$CI_{50}$ < 12,3 µg/ml CRS<br>$CI_{50}$ < 1,7 µg/ml CSS<br>$CI_{50}$ < 2,8 µg/ml CRS |
| *Dunalia brachyacantha*<br>Leaves<br>Raqaypampeños | MeOH | antileishmanian<br>antichagasic | Total Lysis of parasites at 10 µg/ml |
| *Notholoena nivea* var. *flava*<br>Leaves<br>Raqaypampeños | DM | antifungal | *Neurospora crassa* at 6 µg/ml<br>*Trichophyton rubrum*<br>*T. mentagrophytes*<br>*Microsporum cannis* at 125 µg/ml<br>*Candida albicans* at 250 µg/ml |
| *Cavanillesia aff. hylogeiton*<br>Stem bark<br>Tacanas | EtOH<br>DM | antimalarial<br>antimalarial | 100% at 1 µg/ml CSS<br>100% at 1 µg/ml CRS<br>100% at 1 µg/ml CSS<br>100% at 1 µg/ml CRS |
| *Senecio smithioides*<br>Leaves<br>Kallawaya | PE | antimalarial | $CI_{50}$ < 1 µg/ml CSS |

*PE: Petroleum Ether; DM: DichloroMethane; EA: Ethyl Acetate; MeOH: Methanol; EtOH: Ethanol

**CSS: Chloroquine Sensitive Strain ; CRS: Chloroquine Resistant Strain

Table II. Activity indexes of active principles from active plants

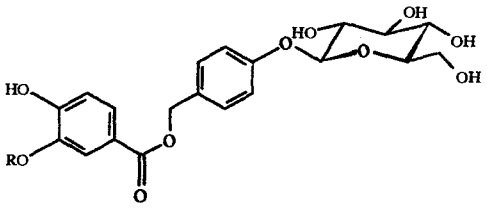
| PLANT | METABOLITE | ACTIVITY |
|---|---|---|
| *Amburana cearensis* (FABACEAE). | **2**. Coumarin | *Plasmodium falciparum* (9.0 µg/ml) *Escherichia coli* et *Shigella flexneri* (actives at 125 µg/ml) |
| | **3**. Amburoside A | *Plasmodium falciparum* (9.1 µg/ml) inactive |
| | **4**. Amburoside B | |
| *Qualea paraensis* (VOCHYSIACEAE) | **5**. 3-β-acetoxyurs-12-en-11-one | *Plasmodium falciparum* (9,0 µg/ml, 21 µM) inactive |
| | **6**. Gult-5-en-3β-yl AcEtate | *Plasmodium falciparum* (6.0 µg/ml,14.5 µM) |
| | **7**. Sitosterol | |
| *Dunalia brachyacantha* (SOLANACEAE) | **8**. 18-acetoxywithanolide D | *Trypanosoma cruzi* (50% epimastigotes, at 50 µg/ml) *Leishmaniae* (total lysis at 25 µg/ml) *Bacillus subtilis* ([+] at 125 µg/ml) *Staphylococcus aureus* ([+] à 62.5 µg/ml) |
| | **9**. 18-acétoxy-5,6-déoxy-5-en withanolide D | *Trypanosoma cruzi* (total lysis at 25 µg/ml) *Leishmaniae* (total lysis at 10 µg/ml) *B. subtilis* ([+] à 125 µg/ml) *S. aureus* ([+] à 62,5 µg/ml) |
| | **10**. Dunawithanine G | inactive |
| | **11**. Dunawithanine H | inactive |
| *Notholaena nivea* var. *Flava* (PTERIDACEAE) | **12**. Pinocembrine | *Neurospora crassa* (13 mm inhibition diameter) inactive |
| | **13**. Pinostrobine | *Neurospora crassa* (13 mm inhibition diameter) inactive |
| | **14**. Sakuranetine | |
| | **15**. 2',6'-dihydroxy-4'-methoxy-dihydrochalcone | |
| *Cavanillesia aff. Hylogeiton* (BOMBACACEAE) | **16**. Lupeol | inactive |
| | **17**. Sitosterol | non tested |
| | **18**. Sitostenone | inactive |
| | **19**. 3-O-β-D-glucopyranosil β-sitosterol | inactive |
| *Senecio smithioides* (ASTERACEA) | **1**. Furanoeremophil-1(10)-en-9-one | *Plasmodium falciparum* (1.2 µg/ml, 5.3 µM) |

**REFERENCES**

1 - PHILLIPSON J. D., WRIGHT C. W., KIRBY G. C., WARHURST D. C. (1995)

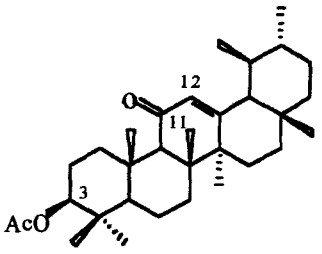
Phytochemistry of some plants used in traditional medicine for the treatment of protozoal diseases, in K. HOSTETTMANN, A. MARSTON, M. MAILLARD, M. HAMBURGER (Eds.), *Phytochemistry of Plants Used in Traditional Medicine* London, Oxford Press, 37: 95-135.

2. WERY M. (1995) *Protozoologie Médicale*, Paris, DeBoeck Université,.
3. ROUSSET J. J. (1995) *Maladies Parasitaires*, Paris, Abrégés Masson,.
4. ATIAS A. (1991) *Parasitologia Clínica*, Mexico D. F., Mediterraneo,.
5. GENTILINI M. (1993), *Médecine Tropicale*, Paris, Flammarion,.
6. BOLHMANN F., ZDERO C., GRENZ M. (1974) Natürlich vorkommende Terpen-Derivative, XXXIX, Über die Inhaltsstoffe der Gattung *Euryops*, *Chem Ber*, 107: 2730-2759.
7. DUPRE S., GRENZ J., JAKUPOVIC J., BOLHMANN F., NIEMEYER H. M. (1991) Eremophilane, germacrene and shikimic acid derivatives from Chilean *Senecio* Species, *Phytochemistry*, 30: 1211-1220.
8. PRETSCH E., SIMON W., SEIBL J., CLERC T. (1989) Tables of Spectral Data for Structural Determination of Organic Compounds, $^{13}$C-NMR, $^{1}$H-NMR, IR, MS, UV/VIS, in W. FRESENIUS, J. F. K. HUBER, E. PUNGOR, G. A. RECHNITZ, W. SIMON, Th. S. WEST, *Chemical Laboratory Practice*, Berlin; Heilderberg, Springer-Verlag, H345.
9. MATSUNAGA S., TANAKA R., AKAGI M. (1988) Triterpenoids from *Euphorbia maculata*, *Phytochemistry*, 27: 535-537.
10. FINUCANE B. W., THOMSON J. B. (1972) Triterpenoids. Part VIII. Allylic oxidation by N-Bromosuccinimide, *J Chem Soc Perkin I*, 1856-1862.
11. BANDARANAYAKE W. (1980) Terpenoids of *Canarium zeylanicum*, *Phytochemistry*, 19: 255-257.
12. YAGISHITA K., NISHIMURA M. (1961) The chemical structure of neoilexonol. I. Some properties of a new triterpenoid ketoalcohol isolated from the bark of *Ilex goshiensis* Hayata, *Agr Biol Chem*, 25; 517-518.
13. RAFFAUF R. F., SHEMLUCK M. J., LE QUESNE P. W. (1991) The withanolides of *Iochroma fuchsioides*, *J Nat Prod*, 54: 1601-1606.
14. JUNG J. H., PUMMANGURA S., CHAICHANTIPYUTH C., PATARAPANICH C., McLAUGHLING. J. L. (1990), Bioactive constituents of *Melodorum fruticosum* *Phytochemistry*, 29: 1667-1670.
15. BAUDOUIN G., TILLEQUIN F., KOCH M. (1983) Isolement, structure et synthèse de la vochysine, pyrrolidinoflavanne de *Vochysia guianensis*, *J Nat Prod*, 46: 681-687
16. BURKE B., NAIR M. (1986) Phenylpropene, benzoic acid and flavonoid derivatives from fruits of jamaican *Piper* species, *Phytochemistry*, 25: 1427-1430.

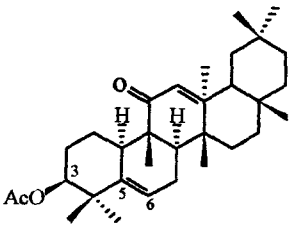


3 amburoside A (R=H)

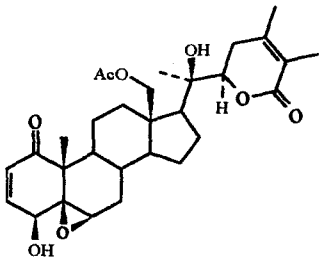
4 amburoside B ($R=CH_3$)



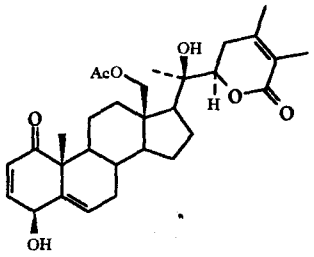
5 3β-acétoxyurs-12-en-11-one



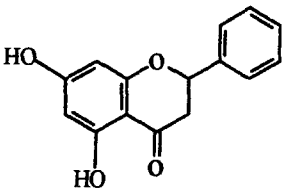
6 acétate de gult-5-en-β-yl



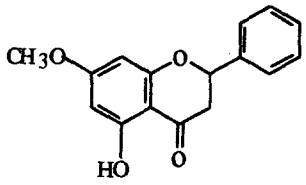
8 18-acétoxywithanolide D



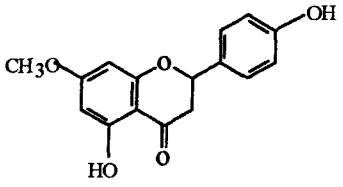
9 18-acétoxy-5,6-déoxy-5-en-withanolide D



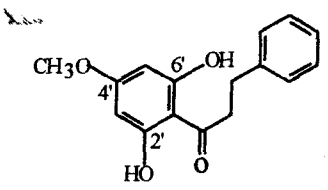
12 pinocembrine



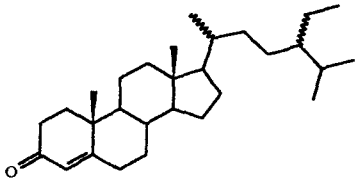
13 pinostrobine



14 sakuranetine



15 2',6'-dihydroxy-4'-méthoxy-dihydrochalcone



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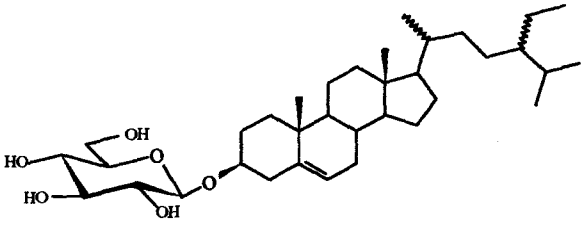
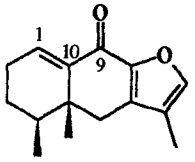


19 3-O-β-D-glucopyranosyl β-sitostérol



1 furanérémophil-1(10)-en-9-one