Pergamon 0031-9422(95)00433-5 Phytochemistry, Vol. 40, No. 4, pp. 1139–1140, 1995

0031 9422/95 $9.50 + 0.00

SESQUITERPENE LACTONES IN *AGROBACTERIUM RHIZOGENES*—TRANSFORMED HAIRY ROOT CULTURE OF *LACTUCA VIROSA*

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(*Received* 2 *February* 1995)

Key Word Index—*Lactuca virosa*; Asteraceae; hairy roots; sesquiterpene lactones; glycosides.

Abstract—Investigation of hairy root culture of *Lactuca virosa* transformed with *Agrobacterium rhizogenes* resulted in the isolation of eight sesquiterpene lactones, six of which were glycoside derivatives. In addition, stigmasterol together with known triterpenes and their acetates were found. The compounds were identified by spectroscopic methods.

INTRODUCTION

Lactuca virosa L. is an old traditional medicinal plant with analgesic, antitussive and sedative properties. These effects have been attributed to the presence of sesquiterpene lactones [1], accumulated mainly in latex, both in roots and aerial parts. The plant reportedly contains the following UV–visible guaianolides: lactucin, its 11β,13-dihydroderivative, lactucopicrin, 8-desoxylactucin (**1**) and jacquinelin (**2**), along with melampolide glucoside lactuside A (**8**) [2, 3].

In a recent communication [4], we described callus and cell suspension cultures of *L. virosa*, which had been established in our laboratory to study the formation of sesquiterpene lactones. However, none of the above mentioned compounds could be detected in the cultures, but three guaianolides not structurally related to lactucin were found.

As an extension of this study we have established a hairy root culture of the plant transformed with *Agrobacterium rhizogenes* LBA 9402 to examine the sesquiterpene lactone composition. Here, we report on the results of this experiment.

RESULTS AND DISCUSSION

The methanol extract of the hairy roots of *L. virosa* was repeatedly chromatographed on silica gel to afford compounds **1**, **2** and **8**, known from roots of the intact plant [2, 3], along with five guaianolide glycosides: crepidiaside B (**3**) [5], picriside A (**4**) [6], macrocliniside A (**5**) [7], ixerin F (**6**) [8] and scorzoside (**7**) [9]. Glycosides of almost the same polarity (**3**, **8** and **5**, **6**, respectively) were separated by HPLC. All the compounds were identified by comparison of their spectra (300 MHz ^{1}H NMR and EI or FAB mass spectra) with those of authentic samples or with literature data. Sesquiterpene lactones and their glycosides, isolated previously in this laboratory from *Lactuca* sp. [2, 3, 10], were used as authentic samples for identification of compounds **1**–**3** and **5**, **6** and **8**. Compound **4** was first reported from *Picris hieracioides* L. var. *japonica* Regel. [6], while compound **7** was isolated from a tissue culture of *Scorzonera hispanica* L. [9].

It should be noted that **6** was found to be a main sesquiterpene lactone component of the extracts from the hairy roots, and the callus and cell suspension cultures [4] of *L. virosa*. The occurrence of this glycoside in plants belonging to the Lactuceae tribe is very common.

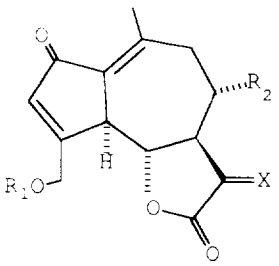
In addition to the sesquiterpene lactones, the hairy roots possess the ability to produce other terpenoids, i.e. stigmasterol and a considerable quantity of widespread pentacyclic triterpenols and their acetates.

EXPERIMENTAL

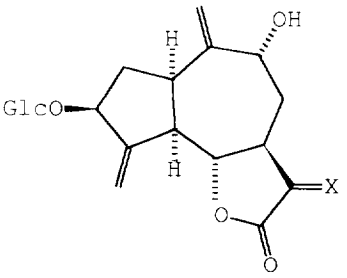
General procedure. Merck silica gel was used for CC (Art. 7754) and TLC (Art. 5553). Semiprep. HPLC was performed on a Delta-Pak C18 cartridge column (particle size 15 μm, 25 × 100 mm) coupled with a UV photodiode array detector. The column was eluted with MeOH–H_2O (7:13) mixt. at a flow rate 3 ml min^{-1}.

Hairy roots. The hairy roots of *L. virosa* transformed with *A. rhizogenes* LBA 9402 were induced in leaf explants from sterile grown seedlings and cultivated in hormone-free Murashige–Skoog liquid medium containing macronutrients reduced to half concn and 3% sucrose, on a gyrotary shaker (110 rpm) in the dark. The roots were harvested after 3 weeks of culture and lyophilized.

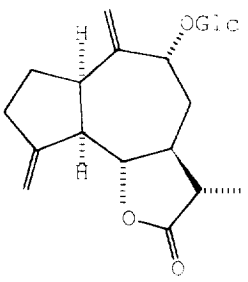
Extraction and isolation. The dried plant material (33 g) was exhaustively extracted with MeOH at room temp. and the residue (8 g) obtained after evapn of the solvent was subjected to CC on silica gel using benzene EtOAc (up to 50%), followed by $CHCl_3$–MeOH (up to 20%) mixt of increasing polarity, as eluents.



	R_1	R_2	X
1	H	H	CH_2
2	H	H	H, α Me
3	Glc	H	H, α Me
4	Glc	OH	CH_2



	X
5	CH_2
6	H, α Me



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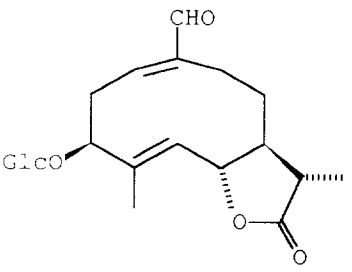

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The low polarity frs eluted with benzene afforded mixts of pentacyclic triterpenols (31 mg, M^+ at *m/z* 426) and their acetates (66 mg, M^+ 468), which were not separated. In the mixts α- and β-amyrin, lupeol and taraxasterol, and their acetates, respectively, could be identified by EI MS and ^1H NMR.

On further elution with benzene–EtOAc (9:1 and 4:1), stigmasterol crystallized (mp 168–169°, 25 mg) with a mixt. of **1** and **2** (7 mg), which was not further sepd.

Elution of the column with $CHCl_3$–MeOH (19:1 and 9:1) mixts gave two crude sesquiterpene lactone glycoside frs. The less polar one was purified by prep. TLC ($CHCl_3$–MeOH, 17:3) giving **7** (5 mg) and **3** (4 mg), and a mixt. of **3** and **8**, which was processed by HPLC to yield an additional amount of **3** (8 mg) and **8** (4 mg). Sepn of the more polar fr. by prep. TLC ($CHCl_3$–MeOH, 17:3, × 3) afforded **4** (6 mg) and a mixt. of **5** and **6**, which was subjected to HPLC, giving 4 mg **5** and 15 mg **6**.

Acknowledgements—We are most grateful to Prof. Dr W. A. Alfermann (Heinrich Heine Universität, Düsseldorf, Germany) for his assistance with aspects of this work. Thanks are also due to the Committee for Scientific Research (KBN) for financial support (grant No. 6 P206 014 04).

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