*Phytochemistry*, Vol. 26, No. 8, pp. 2265–2266, 1987.
Printed in Great Britain.

0031–9422/87 $3.00 + 0.00
Pergamon Journals Ltd.

# ALKALOIDS FROM CALLUS CULTURES OF *HOLARRHENA FLORIBUNDA*

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(*Revised received* 21 *January* 1987)

**Key Word Index**—*Holarrhena floribunda*; Apocynaceae; *in vitro* callus culture; steroidal alkaloids; conessine; GC.

**Abstract**—From callus cultures of *Holarrhena floribunda* a complex mixture of alkaloids has been isolated in small yield, the main alkaloid of which was identified as conessine by MS, GC and TLC.

## INTRODUCTION

Despite the extensive investigations on the alkaloidal constituents of *Holarrhena floribunda* Wall. (Apocynaceae) [1–5], no studies have been carried out on the alkaloids biosynthesized in the callus culture. However, previous studies have shown that tissue cultures of *Holarrhena antidysenterica* produce several steroids and alkaloids of which one was tentatively identified by thin-layer chromatography (TLC) as conessine [6]. In the present report, preliminary results on the production of alkaloids by callus cultures are discussed.

## RESULTS AND DISCUSSION

After the usual extraction procedure of alkaloids from calli cultured on a Gamborg's medium [7], at least eight alkaloids were detected by TLC. The isolation of the main constituent by preparative TLC allows the identification of conessine by TLC, gas chromatography and MS by comparison with an authentic sample. High performance TLC-densitometry with Dragendorff and iodo-platinate as chromogenic reagents [8] was applied to determine conessine and other alkaloids in the extracts. Three strains were investigated: a callus initiated from the stem and cultured either in light conditions or in the dark and a callus initiated from the root cultured in the dark. A comparison of the TLC scanning profile of these three extracts and of known concentrations of conessine allowed the determination of the conessine and the total alkaloids content (Table 1). Conessine proportions reach *ca* 50% of the total alkaloid extracts and are thus comparable to those observed in extracts from stem and root barks [9]. However, the concentration of the total alkaloids obtained in standard experimental conditions of culture is low (0.001% fr. wt). Only quantitative differences appear between the strains; the highest concentrations were found in the one obtained from the root. Light conditions were not favourable to alkaloid production in the calli initiated from the stem; this effect is not measurable in the roots which did not generate callus in the light.

## EXPERIMENTAL

*Plant material.* Seeds of *H. floribunda* were collected in Burkina Faso in October 1985. Voucher specimens of the plant are kept in the Herbarium (N 82–409, 82–520, 83–013 BRLU, BR).

*Callus culture.* Seeds were sterilized in 70% EtOH (5 min) then in 50% NaClO (5 min) and rinsed with sterile $H_2O$. Seeds were germinated on a solid Knop 1/2 medium [10] in the dark at 22°. After 8 to 14 days aseptic germinated seeds were transfered to continuous light (2000 lx) at 25°. Callus from 3-week-old seedling stems were initiated on a complete, solid Gamborg's medium solidified with Siglid agar (1%) and supplemented with 2,4-D (1 ppm) and kinetin (0.1 ppm). The pH of the medium was adjusted to 5.5 with 0.1 M NaOH before autoclaving (110°, 20 min). The cultures were placed at 25° either in the dark or in continuous light (2000 lx). No callus was produced by the root explant exposed to light. All 3 other culture conditions produced calli which were subcultured in the same initial conditions every 30 days.

*Extraction.* After 4 weeks of culture, calli were refluxed in boiled EtOH for 20 hr. After filtration, the EtOH soln was evaporated *in vacuo* to dryness and the residue was suspended in 1 M HCl (50 ml). After filtration, the acidic soln was extracted with $CHCl_3$, then made alkaline with $NH_4OH$, and extracted with $CHCl_3$. The residue of evaporation of this last $CHCl_3$ soln gave the final extract which was redissolved in 0.5 ml $CHCl_3$. 1 $\mu$l of this soln was applied on a silica gel $60F_{254}$ precoated HPTLC plate; the development was performed with EtOAc–$C_6H_{12}$–$Et_2NH$ (75:24:6) and photodensitometry of the chromatograms was carried out after spraying Dragendorff and iodo-platinate reagents.

*Prep. TLC and conessine identification.* An alkaloid extract corresponding to 25 g of callus (fr. wt) was applied to a silica gel plate and after development with the mobile phase used for the analytical method, the alkaloid corresponding to a reference sample of conessine was eluted from the adsorbent with $CHCl_3$–MeOH (1:1). EIMS of conessine was recorded on a VG Micromass 7070F instrument using a direct inlet system and an ionizing energy of 70 eV; *m/z*: 356[m]$^+$, 341[M − 15]$^+$. GC of conessine was performed on a HRGC 5160 Carlo Erba instrument equipped with an OV-1 bonded column (50 m × 0.32 mm); carrier gas He, flow 1 ml/min, oven temp 235°, FID detection at 275°, on column injuction, $R_t$ of conessine, 28.5 min.

Table 1. Concentrations of conessine and total alkaloids content in three strains obtained from *H. floribunda*

| Origin of the strain | Culture conditions | Conessine contents | Content of other alkaloids | Total alkaloid content |
|---|---|---|---|---|
| Stem | dark | 47 | 41 | 88 |
| Stem | continuous light | 35 | 27 | 62 |
| Root | dark | 58 | 52 | 110 |

The concentration are given in μg/10g callus (fr. wt). The total of the other alkaloids separated by TLC are expressed as conessine. The relative standard deviation is evaluated to 10.

*Acknowledgements*—The authors wish to thank Ms G. Vandewode for her generous gift of alkaloid standards, Dr C. Hootele and Mr M. Jaziri for their kind assistance and Prof. J. Lejoly for the collection of seeds. This research is supported by the 'Fonds de la Recherche Fondamentale Collective' (Belgium) Grant 2.4519.86.

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