Phytochemistry, Vol. 27, No. 1, pp. 293–294, 1988.
Printed in Great Britain.

0031-9422/88 $3.00 + 0.00

STIMULATION OF ADVENTITIOUS ROOT FORMATION ON MUNG BEAN CUTTINGS BY COLEON O

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(*Revised received* 15 *May* 1987)

Key Word Index—*Coleus scutellarioides*; *C. blumei*; Lamiaceae; Coleon O; adventitious root formation; mung bean; *Phaseolus aureus*.

Abstract—Coleon O was identified as a rooting factor in extracts from *Coleus scutellarioides* and *C. blumei*. Its identity was confirmed by IR, NMR, MS and GC-MS. It stimulates adventitious root formation on light grown mung bean cuttings by about 100% in concentrations between 2×10^{-4} and 2×10^{-5} M.

INTRODUCTION

While studying the intermediary polar components from extracts of freeze-dried *Coleus* shoots, we found a fraction which stimulated the adventitious rooting of light grown mung bean cuttings by more than 400%. After further purification we were able to identify one of the active compounds as Coleon O, a diterpene previously obtained from *C. somaliensis S. Moore* [1]. Coleons are diterpenes which are found in several species of *Coleus*, *Plectranthus* and *Solenostemon* ie closely related genera of Lamiaceae. They are characterized by an abietane structure (Fig. 1). The accumulation of these compounds is restricted to special glands on leaves, shoots and even flowers of the plants. Sometimes their amount surpasses 1% of the plants dry weight [2].

RESULTS AND DISCUSSION

On analysis of biologically active extracts of *C. scutellarioides Benth.* and *C. blumei Benth*, we were able to isolate and identify Coleon O as one of the active principles. Coleon O was first isolated from *C. somaliensis S. Moore* [1] and later from *C. coerulescens Gürcke* [3], *C. garckeanus Vatke* and *Solenostemon sylvaticus* (*Gürcke*) *T.T. Aye.* [4].

Coleon O stimulated adventitious root formation on light grown mung bean cuttings at concentrations between 2×10^{-4} and 2×10^{-5} M (Table 1). At 2×10^{-4} M the basal 2 mm of the hypocotyl became necrotic, which indicates toxicity at higher concentrations. This is the first time that a physiological effect of Coleon O has been reported. Eugster [5] suggests that the coleons represent a series of intermediates of terpene metabolism.

Heliangine [6] and portulal [7], two terpenes which are known to stimulate adventitious rooting in mung bean cuttings have no structural similarity with Coleon O. Kalsi [8] stated that certain sesquiterpene lactones need an exocyclic methylene conjugated to a lactone carbonyl to be active, but this requirement is not common among biologically active terpenoids.

More study is needed to elucidate how Coleon O is able to stimulate adventitious rooting. As the activity on rooting of the crystalline Coleon O is about three times lower than the activity of the less purified extracts, further research will be done to identify the other active compounds in the crude extract.

EXPERIMENTAL

Plant material. Plants of *C. scutellarioides* and *C. blumei* were grown in a greenhouse. When the plants were fully developed (±50 cm height) the aerial parts were cut off and freeze-dried.

Deinfected mung bean seeds were grown in moist vermiculite at 27°, 70% relative humidity and under continuous TL-light (about 4000 lux). After five days, cuttings with a hypocotyl length of 6 cm were made. After removal of the cotyledons, the cuttings were incubated in 10 ml test soln (10 cuttings per vial) under the same conditions as described above. After five days the adventitious roots were counted. Test solns were made in 0.2% DMF to solubilize Coleon O. Controls received 0.2% DMF.

Isolation of Coleon O. Our preliminary extraction procedure permitted us to obtain a highly purified active fraction, in which we could identify Coleon O by spectral and chromatographic data (see below). After the identification, we used an easier extraction procedure [3] for obtaining crystalline Coleon O for use in the physiological experiments.

500 g freeze-dried shoots were extracted overnight with 10 l Et_2O at room temp. After evapn of the solvent, the residue was dissolved in 500 ml 95% MeOH. Apolar compounds were removed by 6 extractions with equal vols of C_6H_{14}. The MeOH

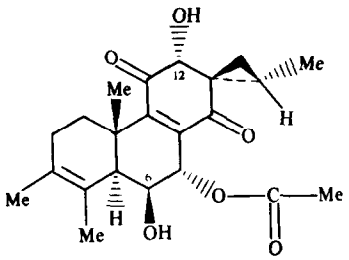

Fig. 1. Structure of Coleon O

Table 1. Rooting activity on light grown mung bean cuttings of Coleon O as compared to a less purified extract (Mean ± s.e. of 3 × 10 cuttings per treatment)

Treatment	M	Mean number of roots per cutting
Coleon O	2×10^{-4}	6.3 ± 0.2
	10^{-4}	8.7 ± 0.8
	8×10^{-5}	8.6 ± 1.4
	6×10^{-5}	7.9 ± 0.9
	4×10^{-5}	5.5 ± 0.3
	2×10^{-5}	5.0 ± 0.3
Crude extract	6 g/l	27.0 ± 1.3
H_2O		4.3 ± 0.2

was evaporated at 35° under red. pres. and the residue (± 500 mg) roughly purified on a 40 g silica gel column which was eluted with 1400 ml C_6H_{14}–Me_2CO (6:1). After evapn of the solvent the residue was transferred onto a polyamide column (10 g) which was eluted with 400 ml MeOH–H_2O (7:13). The MeOH was removed at 35° under red. pres. and the remaining H_2O-phase was freeze-dried. This residue was separated on a 10 g silica gel column which was eluted with 400 ml $CHCl_3$–C_6H_{14}–MeOH (100:100:1). After evaporation of the solvent, the residue was taken up in $CHCl_3$–*iso*-Pr_2O (1:2). Coleon O pptd as colourless needles (20 mg).

UV λ_{max}^{MeOH} nm: 204.3 (0.653), 237.5 (0.424); IR ν_{max}^{KBr} cm^{-1}: 3500 (–OH), 2925 (–CH_3), 1750 (C=O), 1675 (–C(=O)–C=C–C(=O)–), 1020 (cyclopropyl); ^{1}H NMR of 12-*O*-acetyl-Coleon O (Coleon M) (400.1 MHz, $CDCl_3$, TMS as int. stand.): δ 2.43 (1H, H-5), 4.30 (1H, *dd*, H-6), 5.70 (1H, *d*, H-7) 4.80 (1H, *s*, H-12), 2.10 (1H, *m*, H-15), 1.00 (1H, *dd*, H-16), 1.30 (1H, *dd*, H-16), 1.15 (3H, *d*, H-17), 1.78 (3H, *s*, H-18), 1.68 (3H, *s*, H-19), 1.47 (3H, *s*, H-20); MS of Coleon O (70 eV) *m/z* (rel. int.): M^+ not visible, 328 $[M-HOAc]^+$ (100), 313 $[M-HOAc,-Me]^+$ (54), 310 $[M-HOAc,-H_2O]^+$ (66), 295 $[M-HOAc,-H_2O,-Me]^+$ (90); GC: CP^{tm} Sil_5 (WCOT), 240°, H_2 (0.7 bar), Detector temp. 270° (FID), injector temp. 250°. Injection vol 0.2 μl. Because Coleon O is temperature sensitive, it was acetylated (Ac_2O–C_5H_5N, room temp. 1 hr) to make the more stable Coleon M (12-*O*-acetyl-Coleon O). Due to steric hindrance at C-6 only the hydroxyl at C-12 is acetylated [1]. R_t 5.14 min. GC–MS confirmed the identity of Coleon M in the GC chromatogram.

Acknowledgements—The authors wish to thank Dr Compernolle and Dr S. Toppet, Department of Organic Chemistry, University of Leuven, for MS and NMR analysis and Ir J. Czech and Dr L. Martens, University of Diepenbeek, for GC–MS and the Belgian I.W.O.N.L., N.F.W.O. and F.K.F.O. for financial support.

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