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PHYTUBEROL FROM *NICOTIANA RUSTICA* INOCULATED WITH TOBACCO MOSAIC VIRUS

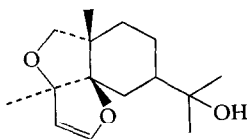
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Key Word Index—*Nicotiana rustica*; Solanaceae; tobacco; sesquiterpenoid stress compound; phytuberol; tobacco mosaic virus.

Many structurally-related sesquiterpenoid stress compounds, such as rishitin and lubimin, have been isolated from the Solanaceae [1, 2]. Capsidiol [3], solavetivone [4, 5], phytuberin [6, 7], phytuberol [7] and glutinosone [8] have been shown to be stress compounds in plants of the genus *Nicotiana*. The first four compounds have been produced in leaves of *Nicotiana tabacum* in response to tobacco mosaic virus (TMV) [4, 5], tobacco necrosis virus (TNV) [3], the bacterium *Pseudomonas lachrymans* [6] or ethrel [7]. Capsidiol has also been found in leaves of *Nicotiana clevelandii* [3] and glutinosone in *N. glutinosa* inoculated with TMV.



1

Here, we report the accumulation of terpenoid stress compound phytuberol (**1**) in leaves of *N. rustica* inoculated with TMV. The CH_2Cl_2 extract from the inoculated leaves contained phytuberol (**1**), which was first suggested by GC-MS analysis. The extract was steam-distilled and subsequently chromatographed on silicic acid column. Phytuberol was isolated from fractions eluted with hexane- Et_2O (1:1) and 100% Et_2O . The concentration of isolated phytuberol was 1.11 mg/g dry wt of inoculated leaves. Mass, IR and ^1H NMR spectra, $[\alpha]_D$, and the retention time of GLC of the isolated phytuberol were in accord with those of an authentic sample [9]. Phytuberol was not detected in healthy leaf by GC-MS analysis.

EXPERIMENTAL

Nicotiana rustica was grown in a greenhouse at 24°. Tobacco plants (2 months old) with 6–8 fully expanded leaves were inoculated with TMV (0.5 $\mu\text{g}/\text{ml}$ in 0.1 M phosphate buffer). Carborundum was used to aid virus infection. When brown lesions had been produced (7 days), the leaves were harvested.

The harvested leaves (396 g fr. wt) were frozen at -20° for 2 days and then freeze-dried. The dried materials (45 g) were extracted with CH_2Cl_2 (1 l. \times 3). The solvent was distilled off from the crude extract, which was then steam-distilled. The distillate (1.5 litre) was saturated with NaCl and extracted with Et_2O . The extract was evapd to dryness to give 230 mg of the volatile. The volatile was introduced onto a column of silicic acid (10 g) and eluted with hexane- Et_2O .

Phytuberol (**1**) was isolated as an oil (50 mg) from fractions eluted with hexane- Et_2O (1:1) and 100% Et_2O . Characteristic retention time on GLC was 10.3 min (5% OV 101 on Chromosorb W (AW), 3 mm \times 1 m, 100–240°, 5°/min, 60 ml He/min).

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GERMACRANOLIDES FROM *ERLANGEA CORDIFOLIA**

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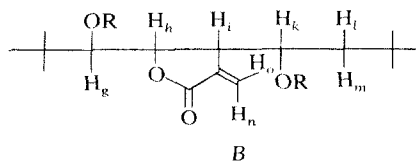
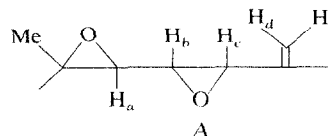
Key Word Index—*Erlangea cordifolia*; Compositae; Vernonieae; new sesquiterpene lactones; germacranolides; triepoxide.

INTRODUCTION

So far only 3 members of the large African genus *Erlangea* (Compositae, tribe Vernonieae) have been investigated. In addition to simple sesquiterpenes, one species contains two guaianolides [1], while in a second one germacranolide of the glaucolide type was found [2]. The third species only contains special 5-methylcoumarins [3] also present in *Bothriocline* [4] and *Ethulia* [5], both being African genera belonging to the Vernonieae. A germacranolide is reported in a patent application from *E. cordifolia* [6], however, no stereochemistry was given. We now report the isolation and structure elucidation of two highly oxygenated germacranolides from the same plant, which are named cordifene and 4,15-epoxy-4,15-dihydro-cordifene.

RESULTS AND DISCUSSION

The polar fractions of the aerial parts of *E. cordifolia* afforded, as the main constituent, a crystalline compound with the molecular formula $C_{20}H_{24}O_7$. The 1H NMR spectrum (Table 1) was very similar to the spectrum reported by Mugo [6]. The 270 MHz 1H NMR spectrum displayed typical signals for a methylene lactone (*s(br)*, 6.31 and 5.67) and those for an angelic acid ester (*qq*, 6.08; *dq*, 1.93 and *dq*, 1.79). Extensive double resonance experiments lead to the sequences A and B.



Irradiation of the signal for H_b (*dd*, 3.00) collapsed the doublet at 2.60 and the triplet doublet at 3.97 to singlets. Irradiation at 3.97 sharpened the signals of the methylene protons at 5.53 and 5.39. The coupling J_{H_b, H_c} was 7.5 Hz indicating a *cis* configuration of 1- and 2-H as in mikanolide [7, 8]. As the signal at 3.60 showed no further couplings and as there was a downfield methyl singlet at 1.58, the position of this group seems to be established. On irradiation of H_h (*d(br)*, 4.38), a doublet at 4.12 H_g changes to a singlet, while irradiation of H_i sharpened the broadened singlets at 6.31 and 5.67 (13-H), and the *ddd* 5.11 became a *dd*. This clearly showed that the signal at 2.92 must be assigned to 7-H. Further irradiation of H_k (*ddd*, 5.11) collapsed the two double-doublets at 2.57 and 1.34 to doublets. These results are only in agreement with part B. However, the relative positions of the oxygen functions remained to be elucidated. On acetylation a monoacetate was obtained. In the 1H NMR spectrum (Table 1) the signal

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