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STRESS COMPOUNDS FROM *NICOTIANA RUSTICA* INOCULATED WITH TMV

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Key Word Index—*Nicotiana rustica*; Solanaceae; tobacco; sesquiterpenoids; stress compounds; occidenol; occidentalol; *trans*-occidentalol.

Abstract—Three new sesquiterpenoid stress compounds, occidenol, occidentalol and *trans*-occidentalol, have been isolated from the leaves of *Nicotiana rustica* inoculated with TMV.

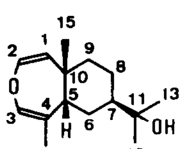
INTRODUCTION

Stress compounds are produced in tobacco leaves in response to virus infection, bacterial infection or ethrel treatment. To date, 16 sesquiterpenoids, solavetivone [1–5], 3-hydroxysolavetivone [2, 5], solanascone [2], phytuberin [2, 4, 6–8], phytuberol [2, 4, 6, 8, 9], glutinosone [10], oxyglutinosone [5], capsidiol [4, 5, 11–13], occidol [14], occidol isomer-1 [14], occidol isomer-2 [14], occidol acetate [14], rishitin [4, 5, 13], epirishitin [5], lubimin [4] and 3-hydroxylubimin [5] have been reported as stress compounds of *Nicotiana* species. Among them, phytuberol is produced as the main stress compound [9] and occidol (**3**), occidol isomer-1, occidol isomer-2 and phytuberin as minor stress compounds [14] in the leaves of *Nicotiana rustica* L. inoculated with tobacco mosaic virus (TMV). Here, we report the isolation and identification of three novel tobacco stress compounds, occidenol (**1**), occidentalol (**2a**) and *trans*-occidentalol (**2b**), from the leaves of *N. rustica* inoculated with TMV. Of these compounds, **2b** is a sesquiterpenoid not known so far in nature. Detailed assignments of ^{13}C NMR signals of **1** and **2a** are given.

RESULTS AND DISCUSSION

Occidenol (**1**) was isolated as a white crystalline substance from a fraction obtained after elution with hexane–ether (4:1) on a silica gel column of the steam distillate of the leaves inoculated with TMV. The mass spectrum of **1** showed $[M]^+$ at m/z 236 corresponding to $C_{15}H_{24}O_2$. A base peak at m/z 59 indicated the presence of an isopropanol group. This was supported by the presence of an absorption band at 3420 cm^{-1} in the IR spectrum and a signal at δ1.27 (6H, *s*) in the ^{1}H NMR spectrum. IR absorption bands due to a vinylic ether were observed at 1670, 1655, 1295, 1195 and 1030 cm^{-1}. The ^{1}H NMR spectrum also contained signals for an angular methyl group (δ1.15, 3H, *s*), a vinylic methyl group (δ1.67, 3H, *d*) and three olefinic methine groups (δ4.40, 1H, *dd* and 6.09, 2H, *d* and *br s*), of which two were adjacent to oxygen. These spectral data were in good agreement with those obtained with authentic occidenol (**1**), which had been reported as one of the constituents of the heartwood of *Thuja occidentalis* [15], but not as a stress compound of infected tobacco leaves. In the ^{13}C NMR spectra, 15 signals were recognized, the multiplicities of which were determined by the INEPT method. Olefinic and oxygen-carrying carbons were assigned by their characteristic chemical shifts. Further assignments were made in lanthanide-induced shift (LIS) experiments. Among four methyl carbon signals, those at δ29.4 and 29.5 (RIS 3.81 and 3.88) were assigned to C-12 or C-13 because the RIS values of the remaining methyl signals at δ31.0 and 21.5 were 0.49 and 0.39. These signals were finally assigned to C-14 and C-15, respectively. The weaker LIS shift (RIS 1.03) of the methylene carbon signal at δ38.4 than those (RIS 1.70 and 1.83) of the methylene carbon signals at δ28.8 and 22.6 indicated that the signal at δ38.4 was due to the C-9 methylene. The signals at δ28.8 and 22.6 were assigned to C-6 and C-8 methylenes by comparison with the chemical shifts of the corresponding methylenes of dihydroeudesmol [16]. Furthermore, the difference between the methine carbon signal at δ48.1 for C-5 (RIS 1.11) and that at δ42.4 for C-7 (RIS 2.47) was explained.

Occidentalol (**2a**) and *trans*-occidentalol (**2b**) were isolated as white crystalline substances from the fraction containing **1**. The mass spectrum of **2a** showed a molecular ion peak at m/z 220, corresponding to $C_{15}H_{24}O$, and a fragment ion peak at m/z 59 indicating the presence of an isopropanol group. Its IR spectrum showed the presence



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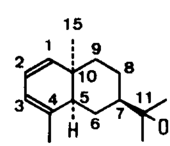
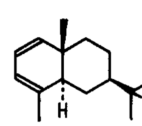
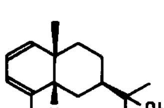


2a



2b



2c

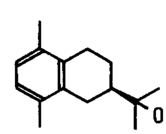


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Table 1. ^{13}C NMR spectral data of **1** and **2a** (25 MHz, $CDCl_3$, TMS as int. standard)

	1			**2a**		
C	δ	INEPT	RIS*	δ	INEPT	RIS*
1	115.5	CH		133.2	CH	
2	140.9	CH		123.5	CH	
3	137.8	CH		116.8	CH	
4	120.2	C		139.8	C	
5	48.1	CH	1.11	47.7	CH	1.20
6	28.8	CH_2	1.70	27.3	CH_2	
7	42.4	CH	2.47	47.3	CH	2.58
8	22.6	CH_2	1.83	24.8	CH_2	
9	38.4	CH_2	1.03	39.1	CH_2	
10	38.2	C		35.7	C	
11	74.1	C	10.0	72.8	C	10.0
12	29.4 or 29.5	Me	3.81	26.9 or 27.1	Me	3.88
13	29.5 or 29.4	Me	3.88	27.1 or 26.9	Me	3.90
14	31.0	Me	0.49	26.0	Me	0.35
15	21.5	Me	0.39	22.2	Me	0.10

*RIS (relative induced shifts) related to the signal assignments are described.

of a hydroxyl group (3300 cm^{-1}) and a conjugated diene (1650, 1590 and 720 cm^{-1}), of which one was trisubstituted (850 cm^{-1}). Furthermore, its ^1H NMR spectrum was consistent with the presence of an isopropanol group (δ1.14, 6H, *s*), a tertiary methyl group (δ0.88, 3H, *s*), a vinylic methyl group (δ1.79, 3H, *s*) and a conjugated diene (δ5.28, 1H, *d*; 5.56, 1H, *m* and 5.82, 1H, *dd*). These spectral data were in accord with those of authentic occidentalol (**2a**). Compound **2a** had also been found in the essential oil of *T. occidentalis* [17, 18]. However, this is the first report of **2a** as a stress compound in inoculated tobacco leaves. The ^{13}C NMR signals were assigned as in the case of **1**. The LIS experiment facilitated the assignments of carbons at C-5, C-7, C-12 and C-13. The mass and IR spectrum of **2b** were very similar to those of **2a**, indicating that **2b** was a stereoisomer of **2a**.

A comparison of the ^1H NMR data substantiated this conclusion and suggested that **2b** is a C-10 epimer of **2a** since the shieldings of the protons at C-12, C-13 and C-15 were found to be markedly different, δ1.14 (C-12, -13) and 0.88 (C-15) for **2a** *cf* δ1.23 and 0.80 for **2b**. These data were in accord with those of *trans*-occidentalol (**2b**) which has been previously reported as a synthetic compound [18]. The amount of **2b** decreased upon storage at −20°. It has been suggested that occidol (**3**) arises in *Thuja* by an efficient conversion of 7-*epi*-occidentalol (**2c**) [19]. This is the first report of the existence of **2b** in nature. Compounds **1**, **2a** and **2b** were not detected in healthy leaves.

EXPERIMENTAL

^1H NMR (100 MHz) and ^{13}C NMR (25 MHz): JEOL FX 100 Fourier transform NMR spectrometer, $CDCl_3$, TMS as int. standard; GC/MS (Hitachi M-80): capillary column (Carbowax 20M fused-silica 0.31 mm × 25 m, 100–210°, 2°/min). GC conditions; prep. GC: 5% FFAP on Chromosorb W(AW) (3 mm × 1 m), 100–240°, 5°/min; capillary GC: PEG 20 M Ultra Bond (0.27 mm × 60 m), 100–210°, 2°/min.

Leaves of *N. rustica* were treated in a similar manner to that described in ref. [2]. The steam distillate was obtained and fractionated in the same manner as described in ref. [14].

Compounds **1**, **2a** and **2b** were isolated by prep. GC.

Occidenol (**1**). $C_{15}H_{24}O_2$ [Found: 236.1741, Calc.: 236.1775]; $[\alpha]_D^{23}$ −121°; mp 35–36°; MS *m/z* (rel. int.): 236 $[M]^+$ (9), 221 (6), 218 (5), 203 (8), 175 (15), 135 (22), 121 (19), 109 (26), 107 (17), 95 (38), 93 (18), 91 (20), 79 (25), 77 (18), 59 (100), 43 (48), 41 (41); IR $\nu_{\max}^{\text{film}}$ cm^{-1}: 3420, 1670, 1655, 1295, 1195, 1030: ^1H NMR: δ1.15 (3H, *s*), 1.27 (6H, *s*), 1.67 (3H, *d*, *J* = 1.1), 2.18 (1H, *m*), 4.40 (1H, *dd*, *J* = 7.6, 1.1), 6.09 (1H, *d*, *J* = 7.6), 6.09 (1H, *br s*); ^{13}C NMR: Table 1.

Occidentalol (**2a**). $C_{15}H_{24}O$ [Found: 220.1788, Calc: 220.1826]; mp 94–96°; MS *m/z* (rel. int.): 220 $[M]^+$ (14), 202 (10), 187 (16), 159 (31), 145 (32), 131 (100), 119 (42), 105 (34), 91 (35), 59 (55), 43 (34), 41 (23); IR $\nu_{\max}^{\text{film}}$ cm^{-1}: 3300, 1650, 1590, 850, 720: ^1H NMR; δ0.88 (3H, *s*), 1.14 (6H, *s*), 1.79 (3H, *s*), 5.28 (1H, *d*, *J* = 9.5), 5.56 (1H, *m*), 5.82 (1H, *dd*, *J* = 5.2, 9.5); ^{13}C NMR: Table 1.

trans-*Occidentalol* (**2b**). ^1H NMR: δ0.80 (3H, *s*), 1.23 (6H, *s*), 1.78 (3H, *d*, *J* = 1.0), 5.5–5.8 (3H, *m*).

The amounts of **1**, **2a** and **2b** were 5, 2 and 2.5 μg/g fr. wt, respectively (estimated by measurement of the peak areas in the capillary gas chromatograms using a Hewlett–Packard 3380A digital integrator).

Characteristic R_ts (min) on capillary GC were **1** 52.4, **2a** 42.1 and **2b** 47.9.

Lanthanide-induced shift expts were carried out using the shift reagent $Eu(fod)_3$ at the mol ratios, 1/30, 1/15, 1/10, 2/15, 1/6, 1/5.

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MARIOLIN, A GERMACRANOLIDE FROM *ANACYCLUS RADIATUS**

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Key Word Index—*Anacyclus radiatus*; Compositae; sesquiterpene lactone; germacranolide; mariolin.

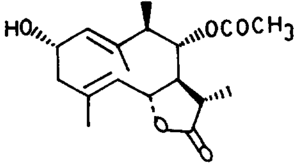
Abstract—A new sesquiterpene lactone, the germacranolide mariolin, was isolated from the aerial part of *Anacyclus radiatus* along with several known compounds. The structure of mariolin was determined by spectroscopic methods.

INTRODUCTION

The genus *Anacyclus* (tribe Anthemideae) with about eight species is mainly distributed in the southern part of the Iberian Peninsula and in North Africa. This genus has not been investigated chemically; therefore we have studied the constituents of *Anacyclus radiatus* Loisel. The aerial parts afforded two flavonoids: isorhamnetin [1] and quercetin [2], a coumarin: isoscopoletin [3], a triterpene: taraxasterol, three sterols: sitosterol, campesterol and stigmasterol, and the new germacranolide mariolin **1**, whose structure was determined from its spectroscopic data.

RESULTS AND DISCUSSION

The ^1H NMR spectrum of mariolin **1** showed a two-proton multiplet at $\delta 4.36$ assigned to geminal hydrogens of allylic hydroxy groups. The presence of an acetate group was confirmed by a three-proton singlet at $\delta 2.05$. A broad singlet (3H) at $\delta 1.89$ and a doublet (3H, $J = 2$ Hz) at $\delta 1.82$ are attributable to the characteristic vinylic methyls of a germacranolide skeleton. A doublet at $\delta 1.31$



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*Part 6 in the series "Structure and Chemistry of Secondary Metabolites from Compositae". For part 5 see González Collado, I., Macias, F. A., Massanet, G. M. and Rodriguez Luis, F., *J. Nat. Prod.* (in press).