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SESQUITERPENOID STRESS COMPOUNDS FROM *NICOTIANA RUSTICA* INOCULATED WITH TMV

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Key Word Index—*Nicotiana rustica*; Solanaceae, tobacco, sesquiterpenoid stress compounds, occidol, occidol isomer-1, occidol isomer-2, occidol acetate, phytuberin, TMV

Abstract—Sesquiterpenoid stress compounds, occidol, occidol isomer-1, occidol isomer-2, occidol acetate and phytuberin have been isolated from the leaves of *Nicotiana rustica* inoculated with TMV. Isomers 1 and 2 and the acetate are sesquiterpenoids not previously reported in nature.

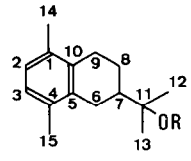
INTRODUCTION

Seven sesquiterpenoids, phytuberin (**4b**) [1–3], phytuberol (**4a**) [2–4], solavetivone [3, 5, 6], 3-hydroxysolavetivone [3] solanascone [3], glutinosone [3, 7] and capsidiol [8], have been found in tobacco leaves as stress compounds We have previously found the production of phytuberol (**4a**) in the leaves of *Nicotiana rustica* inoculated with TMV [4]. We report here the isolation and identification of five sesquiterpenoid stress compounds, occidol (**1a**), occidol isomer-1 (**2**), occidol isomer-2 (**3**), occidol acetate (**1b**) and phytuberin (**4b**), as minor stress compounds from the leaves of *N rustica* inoculated with TMV Compounds **1a**, **1b**, **2** and **3** are new tobacco stress compounds having a tetrahydronaphthalene ring and **2**, **3** and **1b** are new sesquiterpenoids in nature.

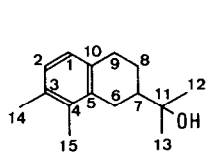
RESULTS AND DISCUSSION

Occidol (**1a**), occidol isomer-1 (**2**) and occidol isomer-2 (**3**) were isolated from the volatiles from leaves inoculated with TMV Capillary GC/MS analysis revealed that these compounds had very similar fragmentation patterns and were present in the ratio of 25.5:1 (**1a**:**2** **3**) The mass spectrum of each compound showed $[M]^+$ at *m/z* 218 corresponding to $C_{15}H_{22}O$. A fragment ion at *m/z* 59 indicated the presence of an isopropanol group and an ion at *m/z* 57 was assumed to be that of protonated dimethylnaphthalene. Preparative GC gave crystalline **3** and a crystalline mixture of **1a** and **2** Compounds **1a** and **2** were separated from each other by capillary GC In the ^{1}H NMR spectrum of the mixture **1a** and **2**, the signals at δ 1 26 (6H, *s*, dimethyls adjacent to OH), 2 19 (6H, *s*, aromatic dimethyls) and 6 88 (2H, *s*, aromatic dimethines) were in accord with those of authentic **1a** The signals of **2** were too small for analysis. The ^{1}H NMR peaks of **3** were measured at δ 1.26 (6H, *s*, dimethyls adjacent to OH), 2 13 (3H, *s*, aromatic mehyl), 2.25 (3H, *s*, aromatic methyl), 6 84 (1H, *d*, aromatic methine) and 6.94 (1H, *d*, aromatic methine) These data suggested that **2** and **3** were methyl rearranged isomers of occidol (**1a**) This was supported by the presence of absorption bands at 3370 (OH), 1600 and 805 cm^{-1} (aromatic) in the IR spectra of both the mixture of **1a** and **2**, and of **3**

The ^{13}C NMR spectrum of the mixture **1a** and **2** showed 13 signals of **1a** and 11 signals of **2** The chemical shifts of the large signals agreed with those of authentic **1a** and the small peaks were considered to belong to its isomer (**2**). The remaining signals of **2** and some of those of **1a** seemed to overlap The assignments of these signals



1a R = H
1b R = Ac



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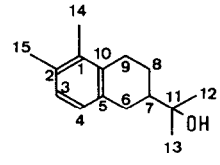

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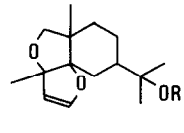
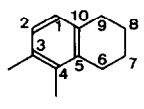


4a R = H
4b R = Ac



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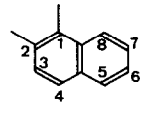

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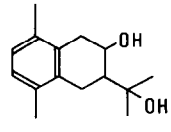

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were confirmed by off-resonance decoupling and lanthanide induced shift (LIS) ^{13}C NMR experiments. The large signals at δ 28 1 (*t*) and 28 8 (*t*) were assigned to C-9 and C-6 of **1a**, respectively, because the LIS of C-6 was greater than that of C-9 on addition of Eu(fod)$_3$ After addition of the shift reagent, the signals of C-14 and C-15 dimethyl carbons, whose chemical shift at δ 19 5 (*q*) was in accord with a signal of dimethyls in 1,4-dimethylnaphthalene [9], separated into two peaks

In the ^{13}C NMR spectrum of the minor compound (**2**), aromatic dimethyls were measured at δ 14.9 (*q*) and 20 4 (*q*) These signals were assigned to C-4 and C-3 methyls, respectively, of partial structure **5**, because the chemical shifts of 1,2-dimethylnaphthalene (**6**) were δ 14.4 for the C-1 methyl and 20 6 for the C-2 methyl [9] In the ^{13}C NMR spectrum of a 3,4-dimethyltetrahydronaphthalene (**5**) the signal from C-9, which is further removed from the methyl groups than C-6, was expected to appear at lower field than C-6 Similarly, in 1,2-dimethylnaphthalene (**6**) C-5 resonates at lower field than C-8 [9] Hence, the signal at δ 30 7 (*t*) in **2** was assigned to the C-9 methylene of partial structure **5** Furthermore, the LIS of this signal was weaker than that of the C-6 methylene of **5**, which indicated that the position of the isopropanol group was at C-7 of **5** These results confirmed the structure of isomer-1 to be **2** (1,2, 3,4-tetrahydro-α,α,7, 8-tetramethyl-2-napthalenemethanol)

Similarly, occidol isomer-2 was assigned structure **3** (1,2,3,4 -tetrahydro-α,α,5,6-tetramethyl-2-naphthalenemethanol) The ^{13}C NMR signals at δ 14.9 (*q*) and 20 3 (*q*) showed the existence of a 1,2-dimethyl aromatic ring. As the methylene carbon furthest removed from the methyl groups at δ 31 6 (*t*) gave rise to a stronger LIS shift, it was assumed that the isopropanol group was located at C-8 of structure **5**

Compound **1b** was isolated from the volatiles as an oil. The mass spectrum of this compound resembled that of occidol (**1a**), **2** and **3** except for the absence of peaks at *m/z* 59 and 218 and the presence of a peak at *m/z* 44 The GC-FI mass spectrum showed a $[M]^+$ at *m/z* 260 corresponding to $C_{17}H_{24}O_2$ These data suggested that this compound was the acetate of occidol This assumption was supported by the presence of an acetate absorption band at 1730 cm^{-1} in the IR spectrum of **1b** In the ^1H NMR spectrum, the signals of the C-12 and C-13 methyl groups underwent a marked downfield acylation shift to δ 1 54 (cf δ 1.26 of **1a**) and the signal of the acetate methyl was observed at 1 99 Similarly, the ^{13}C NMR signal of C-11 shifted downfield and two carbon signals of the acetyl group (C-16 and C-17) appeared at δ 170 3 and 22 4 The structure of this compound was confirmed by direct comparison with a synthetic sample Acylation of occidol with isopropenyl acetate in ether gave occidol acetate. The spectral data of occidol acetate was in agreement with those of **1b** Compound **4b** was identified by capillary GC and capillary GC/MS analyses The mass spectrum and retention time were consistent with those of authentic phytuberin (**4b**).

These compounds were not detected in healthy leaves Compounds **1a**, **1b**, **2** and **3** have a dimethyl benzene ring in common. It may represent these sesquiterpenoids being rearranged from eremophilane- or eudesmane-type sesquiterpenes A similar sesquiterpenoid, rishitinol (**7**), has been reported as a stress compound of white potato tubers inoculated with fungi [10] Compound **1b** has been implicated as an intermediate in the conversion of emmotin-F into **1a** [11]. Compound **1a** has been reported as an essential constituent of *Thuja occidentalis* [12]

EXPERIMENTAL

^1H NMR (100 MHz) and ^{13}C NMR (25 MHz) $CDCl_3$ with TMS as an int standard in a JEOL FX100 Fourier transform NMR spectrometer, GC/MS capillary column (PEG 20M Ultra Bond 0 27 mm × 60 m, 100–210°, 2°/min) in a Hitachi M-80, prep GC 5% FFAP on Chromosorb W (AW), 3 mm × 1 m, 100–240°, 5°/min, capillary GC PEG 20M Ultra Bond 0 27 mm × 60 m, 100–210°, 2°/min

Isolation of **1a**, **1b**, **2**, **3** *and* **4b** Leaves of *N rustica* were treated in a similar manner to that described in ref [3] The virus used

Table 1 ^{13}C NMR spectral data of **1a**, **1b**, **2** and **3** (25 MHz, $CDCl_3$, TMS as an int standard)

C No	1a*	2*	3	1b
1	135 0 (*s*)	125 9 (*d*) or 127 1 (*d*)	134 3 (*s*) or 134 9 (*s*)	134 7 (*s*) or 134 9 (*s*)
2	126 6 (*d*) or 126 8 (*d*)	127 1 (*d*) or 125 9 (*d*)	134.9 (*s*) or 134 3 (*s*)	126 7 (*d*) or 126 9 (*d*)
3	126.8 (*d*) or 126.6 (*d*)	134 9 (*s*)	126 6 (*d*) or 127 2 (*d*)	126 9 (*d*) or 126 7 (*d*)
4	135 0 (*s*)	134 9 (*s*)	127.2 (*d*) or 126 6 (*d*)	134 9 (*s*) or 134 7 (*s*)
5	133 6 (*s*) or 134 1 (*s*)	133 5 (*s*) or 134 2 (*s*)	133 5 (*s*) or 134 5 (*s*)	133 5 (*s*) or 133 9 (*s*)
6†	28 8 (*t*)	28 9 (*t*)	31 6 (*t*)	28 3 (*t*)
7	45 4 (*d*)	46 3 (*d*)	45 3 (*d*)	43 2 (*d*)
8	24 0 (*t*)	—	24.8 (*t*)	23 8 (*t*)
9†	28.1 (*t*)	30 7 (*t*)	28 2 (*t*)	28 0 (*t*)
10	134 1 (*s*) or 133 6 (*s*)	134 2 (*s*) or 133 5 (*s*)	134 5 (*s*) or 133 5 (*s*)	133 9 (*s*) or 133 5 (*s*)
11	72 8 (*s*)	72 7 (*s*)	72 7 (*s*)	84.6 (*s*)
12	26 6 (*q*) or 27.3 (*q*)	—	26 5 (*q*) or 27 3 (*q*)	23 2 (*q*) or 23 3 (*q*)
13	27 3 (*q*) or 26 6 (*q*)	—	27 3 (*q*) or 26 5 (*q*)	23 3 (*q*) or 23 2 (*q*)
14	19 5 (*q*)	20.4 (*q*)	14.9 (*q*)	19 6 (*q*)
15	19 5 (*q*)	14 9 (*q*)	20 3 (*q*)	19 6 (*q*)
16	—	—	—	170 3 (*s*)
17	—	—	—	22 4 (*q*)

*C-8, C-12 and C-13 signals of **2** overlapped with those of **1a**

†Relative induced shifts of C-6 and C-9 (**1a**, **2** and **3**), **1a**. C-6, 1.83, C-9, 0.94; **2** C-6, 1 95, C-9. 0 93, **3** C-6, 2 07, C-9, 0 93 (C-11, 10 0)

was TMV-OM (1.0 μg/ml in 0.1 M Pi buffer, pH 7.0). Local lesion bearing leaves (800 g) gave 111 g dried materials, 6.4 g condensate and 889 mg volatiles. The volatiles were introduced onto a column of silicic acid (15 g) and eluted with hexane–Et_2O.

Compounds **1a**, **2** and **3** were isolated from fractions eluted with hexane–Et_2O (5:1) and (1:1) by prep. GC. MS of **1a**, **2** and **3** *m/z* (rel. int.): 218 $[M]^+$ (5), 200 (26), 185 (37), 159 (23), 157 (100), 145 (21), 119 (26), 59 (53). Mixture **1a** and **2** IR ν_{max}^{film} cm^{-1} 3370, 1600, 805; ^1H NMR. δ 1.26 (6H, *s*), 2.19 (6H, *s*), 6.88 (2H, *s*), ^{13}C NMR. Table 1. Compound **3**: IR ν_{max}^{film} cm^{-1}: 3370, 1600, 805, ^1H NMR: δ 1.26 (6H, *s*), 2.13 (3H, *s*), 2.25 (3H, *s*), 6.84 (1H, *d*, J = 8 Hz), 6.94 (1H, *d*, J = 8 Hz); ^{13}C NMR. Table 1.

Compound **1b** was isolated from the fractions eluted with hexane–Et_2O (19:1) and (9:1) by prep. GC. MS *m/z* (rel. int.). 200 $[M-18-42]^+$ (38), 185 (53), 158 (16), 157 (100), 143 (17), 132 (21), 128 (16), 44 (42); IR ν_{max}^{film} cm^{-1}. 1730, 1600, 805, ^1H NMR. δ 1.54 (6H, *s*), 1.99 (3H, *s*), 2.18 (3H, *s*), 6.89 (2H, *s*); ^{13}C NMR. Table 1.

Compound **4b** was recognized in the fraction eluted with hexane–Et_2O (4:1) by capillary GC and capillary GC/MS.

The amounts of **1a**, **2**, **3**, **1b** and **4b** were 28, 5, 1, 5 and 4 μg/g fr. wt (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 **1a** 68.7, **2** 69.3, **3** 63.1, **1b** 66.9 and **4b** 56.0 min.

Lanthanide induced shift expts were carried out using the shift reagent Eu $(fod)_3$ at the mol ratios, 0.025, 0.05, 0.075, 0.1, 0.125, 0.15, 0.175 and 0.2.

Preparation of occidol acetate (**1b**). Isopropenyl acetate (270 mg) was added to **1a** (500 mg) in BF_3–Et_2O (10 ml). The mixture was stirred at room temp. for 2 hr and then poured into H_2O and extracted with Et_2O. The Et_2O extract was washed with satd $NaHCO_3$ soln and satd NaCl soln, dried and evaporated *in vacuo*. The residue was chromatographed over Si gel (10 g) in hexane–Et_2O. Elution with hexane–Et_2O (19:1) gave 210 mg (42%) of **1b**.

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