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ISOLATION OF (−)-1,2-DEHYDRO-α-CYPERONE AND SOLAVETIVONE FROM *LYCIUM CHINENSE*

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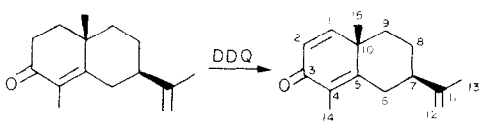
Key Word Index—*Lycium chinense*; Solanaceae; sesquiterpenes; (−)-1,2-dehydro-α-cyperone; solavetivone.

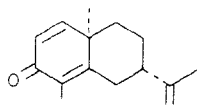
Abstract—The investigation of volatile components of *Lycium chinense* afforded (−)-1,2-dehydro-α-cyperone and solavetivone. (−)-1,2-Dehydro-α-cyperone is a new naturally occurring sesquiterpene and its absolute configuration was determined.

Lycium chinense is widespread in Japan, China, Taiwan and Korea. The half-dried berries, which are used in herbal medicine, have a characteristic odor, although so far little is known on the odor components. We have now investigated the steam-volatile components of the half-dried berries of *Lycium chinense* to isolate two sesquiterpenes, one of which is a new natural product.

Repeated purification by CC and GC of the neutral volatile oil from the air-dried berries afforded two colourless oily compounds (**1** and **2**). Compound **1** showed mass spectral peaks at *m/z* 216[M]$^+$ (4), 201(24), 188(7), 173(34), 159(28), 145(40), 105(80), 91(100), 79(50), 77(33), 67(51), 65(45) and 41(59). By high resolution mass spectrometry the formula of **1** was estimated as $C_{15}H_{20}O$. The IR spectrum showed an α,β-α′,β′-unsaturated carbonyl group at 1658 cm^{-1} and a terminal methylene group at 886 cm^{-1}. The NMR spectrum revealed *cis*-fused olefinic protons [δ 6.76 (1H, *d*) and 6.24 (1H, *d*, *J* = 10 Hz)], an isopropenyl group [4.82 (2H, *m*) and 1.80 (3H, *s*)] and another vinyl methyl group [1.92 (3H, *s*)]. The sharp singlet at δ 1.25 (3H) was considered to show an allylic angular methyl group. In the UV spectrum, **1** showed $\pi \rightarrow \pi^*$ transition at 238 nm (ε = 4600) and 264 nm (ε = 3400) and $n \rightarrow \pi^*$ transition at 313 nm (ε = 52) which were characteristic for an enone system. From these results, a tentative structure 1,2-dehydro-α-cyperone, 5,6,7,8-tetrahydro-1,4a-dimethyl-7-(1-methylethenyl)-2(4a*H*)-naphthalenone was deduced for **1**. To confirm the structure, (+)-α-cyperone (**3**) was dehydrogenated with DDQ. The synthetic material exhibited IR, NMR, UV and mass spectra exactly identical with those of natural **1**. The GC co-injection of the natural and synthetic materials on two different capillary columns (PEG-20M and OV-101) gave a single peak, respectively. The above results indicate that the natural product has the same relative configuration with the synthetic one with respect to C-7 and C-10 (**1a** or **1b**, Scheme 1). The ORD of the natural and synthetic **1** exhibited negative Cotton effects. Thus, the stereostructure of natural **1** is established as 4a*S*, 7*R*-5,6,7,8-tetrahydro-1,4a-dimethyl-7-(1-methylethenyl) - 2(4a*H*)-naphthalenone (**1a**, Scheme 1). This compound has been synthesized by Piers and Cheng [1] and this is the first report of its natural occurrence. Compound **2** showed MS peaks at *m/z* 218[M]$^+$ (50), 203(33), 190(48), 176(70), 161(55), 147(60), 133(59), 120(50), 108(100), 93(48) and 68(47). The NMR gave signals at δ 0.99 (3H, *d*, *J* = 7 Hz), 1.95 (3H, *d*, *J* = 1.2 Hz), 1.76 (3H, *brs*) and 5.73 (1H, *brs*). The GC retention time and spectral data were in accord with those of an authentic sample of solavetivone. Solavetivone has been isolated for the first time as a phytoalexin of potato tuber [2] and later as a minor volatile component of tobacco leaves [3].



3 **1a**



1b

EXPERIMENTAL

Commercially available half-dried berries were re-dried at 55° for 4 hr. The air-dried berries (1.42 kg) were extracted twice with 2 l. CH_2Cl_2 at room temp. for 24 hr to give 163 g of the extract. Neutral oil (110 g) was obtained by ordinary fractionation. It was then steam-distilled and the volatile oil (704 mg) was chromatographed on Si gel using *n*-hexane–Et_2O. 1,2-Dehydro-α-cyperone (**1**, 0.08 mg) and solavetivone (**2**, 0.05 mg) were isolated as colourless oils from the fraction eluted with *n*-hexane–Et_2O(9 : 1). Characteristic GC reten-

tion times for **1** and **2** were 61.6 and 63.2 min on an OV-101 glass capillary column (50 m × 0.27 mm i.d.; 100–240°, 2°/min; carrier gas: He, 0.5 ml/min) and 56.8 and 58.8 min on a PEG-20M fused Si capillary column (50 m × 0.20 mm i.d.; 100–210°, 2°/min; carrier gas: He, 0.5 ml/min), respectively.

(−)-1,2-*Dehydro-α-cyperone* (**1**). Colourless oil; GC/MS 70 eV, *m/z* (rel. int.): 216.1532[M]$^+$ ($C_{15}H_{20}O$, 4%), 201(24), 188(7), 173(34), 159(28), 145(40), 105(80), 91(100), 79(50), 77(33), 67(51), 65(45) and 41(59); IR ν_{max}^{film} cm^{-1}: 1658, 1629, 1603, 886, 829; ^{1}H NMR(100 MHz, $CDCl_3$): δ 6.76(1H, *d*, *J* = 10 Hz, H-2), 6.24(1H, *d*, *J* = 10 Hz, H-1), 4.82(2H, *m*, $W_{1/2}$ = 3 Hz, H-12), 1.92(3H, *s*, H-14), 1.80(3H, *s*, H-13), 1.25(3H, *s*, H-15); UV λ_{max}^{MeOH} nm (log ε): 238(3.66), 264(sh, 3.53), 312(1.72); $[\alpha]_D$ − 172.4°(MeOH; *c* 2.9×10^{-3}); ORD: $[\phi]_{234}$ + 5268, $[\phi]_{265}$ 0, $[\phi]_{281}$ − 1580.

Preparation of (−)-1,2-*dehydro-α-cyperone*. According to the method described [1], (+)-*α*-cyperone (**3**) was reacted with DDQ to give (−)-1,2-dehydro-α-cyperone(**1a**) as colourless needles after purification by prep. GC. The IR, NMR, UV and MS data and the GC retention time were identical with those of natural **1**. $[\alpha]_D$ − 144° (EtOH; *c* 3.40×10^{-2}); ORD: $[\phi]_{233}$ + 14 294, $[\phi]_{264}$ 0, $[\phi]_{280}$ − 9212.

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ACCUMULATION OF SIX SESQUITERPENOID PHYTOALEXINS IN TOBACCO LEAVES INFILTRATED WITH *PSEUDOMONAS LACHRYMANS**

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Key Word Index—*Nicotiana tabacum*; Solanaceae; sesquiterpene stress metabolites; phytoalexins.

Abstract—Tobacco leaves inoculated with *Pseudomonas lachrymans* accumulated capsidiol, rishitin, lubimin, solavetivone, phytuberin and phytuberol.

Six bicyclic sesquiterpenes are established as stress metabolites of *Nicotiana* spp.: Capsidiol in *N. tabacum* and *N. clevelandii* foliage infected with TNV [1] or *P. tabacina* [2] or callus tissue infected by *Phytophthora parasitica* var. *nicotianae* [3]; glutinosone in *N. glutinosa* foliage infected with TMV [4]; rishitin in *N. tabacum* callus tissue infected with *P. parasitica* var. *nicotianae* [5]; phytuberin and phytuberol in *N. tabacum* foliage infected with *Pseudomonas lachrymans* [6] or treated with Ethrel [7]; and solavetivone in *N. tabacum* foliage infected by TNV [8]. In this paper we report the accumulation of the sesquiterpene stress metabolites (SSM) capsidiol, rishitin, lubimin, solavetivone, phytuberin and phytuberol (identified by TLC (Table 1), GC and GC/MS) as a function of time after inoculation of tobacco leaves with *Pseudomonas lachrymans*. Unlike studies with pepper fruit [9] and potato tuber [10] in which maximum accumulation of SSM was reported 48–96 hr after infection, maximum accumulation in response to infection with *P. lachrymans* in tobacco occurred 12–24 hr after infection (Fig. 1). Although a 19 hr lag was observed for rishitin accumulation in potato tuber [11], the data in this paper and in ref. [9] indicate that SSM accumulation can be detected as early as 6 hr after infection. The rapid maximum in SSM accumulation in tobacco foliage may explain the inability to detect appreciable quantities of SSM in foliage of potato and tomato 24 hr or longer after infection with incompatible races of *Phytophthora infestans* or *Cladosporium fulvum*, respectively.

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