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Phytochemistry, 1979, Vol. 18, pp. 873 874. © Pergamon Press Ltd. Printed in England. 0031-9422/79/0501-0873 $02.00/0

MYOPORONE AND RELATED KETO ALCOHOLS FROM STRESSED SWEET POTATOES

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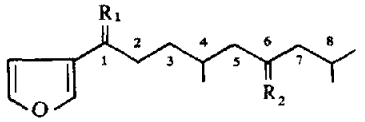
(*Received* 11 *October* 1978)

Key Word Index—*Ipomoea batatas*; Convolvulaceae; sesquiterpene; stress metabolite.

Our investigation of stress metabolites of the sweet potato has led to the isolation of myoprone (**1**) and two related keto alcohols (**2** and **3**) from this plant. The compounds were obtained from a methanol–chloroform extract of mercuric chloride treated sweet potatoes by a combination of column chromatography on Si gel and HPLC. By this method 39 mg of **1**, 109 mg of **2** and 53 mg of **3** were isolated from each kg of treated sweet potatoes.



1 $R_1 = R_2 = O$
2 $R_1 = O$; R_2 = H, OH
3 R_1 = H, OH; $R_2 = O$

Myoporone was initially isolated from Si gel chromatography as a mixture with ipomeamarone. The mixture was separated by HPLC on a reversed phase column. Compound **1** $[\alpha]_D^{25}$ −8.5°, c = 1.54, MeOH) was identified by comparing its ^{1}H NMR and mass spectra to those reported by Blackburne *et al.* [1].

The isomeric keto alcohols were isolated from the Si gel column as a mixture with 7-hydroxymyoporone [2]. This mixture was also resolved by HPLC on a reversed phase column. Compound **2** contained a keto–furan moiety as evidenced by the UV λ_{max} (MeOH) 251 nm (ε = 3000), IR ν_{max} (neat) 1670 cm^{-1}, and the MS fragments at *m/e* 95 and 110 corresponding to cleavage of C-1, C-2 and McLafferty cleavage of C-2, C-3. The broad absorption from 3700 to 3100 cm^{-1} in the IR verified the presence of a hydroxyl group. The MS fragment at *m/e* 195 is consistent with placing the hydroxyl group at C-6. The ^{1}H NMR spectrum of the compound is consistent with structure **2**: δ 0.91 and 0.93 (9H, superimposed *d*, CH_3), 1.34 (4H, *m*, 5- and 7-CH_2), 1.45–2.2 (5H, *m*), 2.80 (2H, *t*, J = 7 Hz, 2-CH_2), 3.88 (1H, *m*, 6-CH), 6.80 (1H, *m*, 4-furyl), 7.46 (1H, *m*, 5-furyl) and 8.07 (1H, *m*, 2-furyl). The product of oxidation of **2** with pyridinium had chlorochromate [3] ^{1}H NMR and mass spectra identical to those of **1**.

Unlike most of the furanosesquiterpenoid stress metabolites from sweet potatoes, C-1 in compound **3** ($[\alpha]_D^{25}$ −6.4°, c = 1.34, MeOH) is not in the ketone oxidation state. This is obvious from the coincidence of signals in the ^{1}H NMR for the 2- and 5-furyl protons, as is seen in the case of 1-ipomeanol and 1,4-ipomeadiol [4], and from the major fragment in the MS at *m/e* 97. The carbonyl absorption at 1705 cm^{-1} in the IR spectrum indicates that the carbonyl group is unconjugated. The fragment in the MS at *m/e* 195 resulting from cleavage at C-6, C-7 and the fragment at *m/e* 100 resulting from McLafferty cleavage of C-4, C-5 places the carbonyl group at C-6. The ^{1}H NMR of **3** is consistent with the proposed structure: δ 0.90 (9H, *d*, J = 7 Hz, CH_3), 1.34 (2H, four line *m*, 3-CH_2), 1.5–2.2 (CH, *m*), 2.26 (4H, *m*, 5- and 7-CH_2), 4.66 (1H, *t*, J = 7 Hz, 1-CH), 6.42 (1H, *m*, 4-furyl) and 7.42 (2H, *m*, 2- and 5-furyl). Oxidation of **3** with pyridinium chlorochromate [3] gave **1** as the major product.

Myoporone has been isolated from *Myoporum bontioides* A. Gray [5], from other *Myoporum* species and *Eremophila* species [1], and from *Eumophia sericea* and *E. prostata* [6]. Thus, myoporone joins epingaione [7] (ipomeamarone [8]), dehydroepingaione [7] (dehydroipomeamarone [9]), and athanagrandione [10] (4-hydroxymyoporone [11]) as furanosesquiterpenes which are stress metabolites of the sweet potato and are also normal secondary metabolites of other plants.

Toxicity studies on compound **2** indicate that it is

hepatotoxic, 2–3 times more potent than ipomeamarone. A detailed report of the toxicity of **2** is forthcoming.

EXPERIMENTAL

NMR spectra were obtained at 100 MHz in $CDCl_3$. MS were run via direct probe inlet at 70 eV. IR spectra were obtained as neat samples. HPLC was carried on a Waters Associates instrument using two 30 cm C-18 μbondapak columns. Column chromatography was carried out using the so-called short column technique of Hunt and Ribgy [12].

Sweet potato roots, cut into 0.5 cm slices, were dipped into a 1% soln of $HgCl_2$ and placed in covered pans for 1 week. At this time the slices were homogenized with MeOH and $CHCl_3$. The $CHCl_3$ extract was dried ($MgSO_4$) and concd *in vacuo*. The residue was chromatographed on a 5 × 7 cm column of Merck Sigel G(Type 60) for TLC. For the isolation of myoporone, 1:5 EtOAc–hexane was used as the eluent: for isolation of **2** and **3**, 1:1 EtOAc–hexane was used.

Myporone was isolated using the C-18 column with 2:1 MeOH–H_2O at a flow rate of 1.7 ml/min as the eluent. Compounds **2** and **3** were isolated by HPLC using the C-18 column with 3:2 MeOH–H_2O at 1.7 ml/min as the eluent. MS of **2**: *m/e* (rel. int.) 252 [M^+] (3), 195 [cleavage at C-6, C-7] (4), 177 [195 $-H_2O$] (9), 166 [cleavage at C-5, C-6] (11), 149 [cleavage at C-4, C-5] (11), 123 [cleavage at 3-C, C-4] (20), 110 [McLafferty at C-2, C-3] (64) and 95 [cleavage at C-1, C-2] (100). MS of **3**: *m/e* (rel. int.) 252 [M^+] (91), 234 [$M^+ - H_2O$] (8), 195 [cleavage at C-6, C-7] (11), 177 [195 $-H_2O$] (8), 127 [cleavage at G3, C-4] (100), 100 (McLafferty at C-4, C-5] (32) and 97 [cleavage at C-1, C-2] (83).

Acknowledgement—This work was supported by Center in Toxicology Grant ES00267 to Vanderbilt University from the U.S. Public Health Service.

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Phytochemistry, 1979, Vol. 18, pp. 874–875. © Pergamon Press Ltd. Printed in England. 0031-9422/79/0501-0874 $02.00/0

ISOLATION AND IDENTIFICATION OF PHYTUBERIN FROM *NICOTIANA TABACUM* PREVIOUSLY INFILTRATED WITH AN INCOMPATIBLE BACTERIUM*

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(*Received* 11 *October* 1978)

Key Word Index—*Nicotiana tabacum*; Solanaceae; incompatible bacteria; sesquiterpene; phytuberin; stress metabolite.

Twenty structurally-related sequiterpenes have been isolated and characterized from Solanaceous species [1, 2]. Three of these compounds, rishitin, lubimin and capsidiol, have been found in more than one species after stress. To date, only capsidiol has been shown to be a stress metabolite in *Nicotiana tabacum* L. [3, 4]. In this paper, we report the isolation of phytuberin, a sesquiterpene previously only reported in potato [5, 6], from tobacco leaf tissue infiltrated with the bacterium *Pseudomonas lachrymans*, a nonpathogen of tobacco.

The presence of phytuberin in extracts from infected tobacco tissue was first suggested by TLC analysis which revealed a compound that had the same R_f and produced the same characteristic color as authentic phytuberin with the vanillin–H_2SO_4 reagent [5]. The unknown was determined to be phytuberin by direct comparison of the unknown with an authentic sample [5], by TLC, GLC, chemical ionization MS and IR. Phytuberin was not detected in healthy leaf tissue.

EXPERIMENTAL

Leaves of 6- to 10-week-old *Nicotiana tabacum* L., cv KY 16, were infiltrated with H_2O or a suspension of the incompatible bacterium *Pseudomonas lachrymans* (10^8 cells/ml) prepared from 24-hour-old cultures. The leaves which were infiltrated

* Journal paper 78-11-153 of the Kentucky Agricultural Experiment Station, Lexington, KY 40506, U.S.A.