(13.5 mg) were rechromatographed on Si gel (8 g) using a gradient of $CHCl_3$, $CHCl_3$–MeOH (98:2–9:1) and MeOH. Twenty fractions of 25 ml each were collected, and **2** hydrochloride (6 mg, 0.008% yield, mp 194–196°) was crystallized (EtOH–Et_2O) from fractions 9–11.

Synthesis of dehydroheliamine (**2**) [4]. The free base (10.17 g) of 3,4-dimethoxyphenethylamine (Aldrich) was refluxed for 16 hr with 10 ml 99% HCOOH. The reaction mixture was poured into 200 ml cold H_2O and extracted with C_6H_6 (5 × 100 ml). The C_6H_6 extract was dried and reduced to an oil (7.77 g). The oil was cooled over ice, 5 ml $POCl_3$ was added, and the mixture was refluxed for 45 min. After cooling, 50 ml petrol was added and then decanted, and 50 ml 10% HCl added. The resultant soln was basified to pHs 7, 8 and 9 with NH_4OH and extracted at each increment with C_6H_6 (100 ml). The C_6H_6 layers were dried and reduced to a brown oil (5.17 g, 48% yield).

This oil then yielded crystalline dehydroheliamine hydrochloride (EtOH–Et_2O) (**2**) (4.32 g). Mp 195–196°; ^{1}H NMR (D_2O, 80 MHz): δ9.13 (1H, *br s*, H-1), 7.81 (1H, *s*, H-8), 7.51 (1H, *s*, H-5), 4.47–4.2 (6H, 2*s*, OMe-2 overlapped with 2H, *m*, H-3), 2.56 (2H, *br t*, H-4); free base: ^{1}H NMR ($CDCl_3$, 80 MHz): δ9.05 (1H, *br s*, H-1), 7.43 (1H, *s*, H-8), 6.78 (1H, *s*, H-5), 4.00–3.82 (6H, 2*s*, OMe-2 overlapped with 2H, *m*, H-3), 3.00 (2H, *br t*, H-4); CIMS *m/z* 192 $[MH]^+$; EIMS *m/z* (%): 191 (100), 190 (15), 176 (70), 164 (5), 146 (10), 136 (30), 117 (12), 104 (15), 91 (10), 77 (20). The synthetic and isolated **2** hydrochlorides were essentially identical: mmp 195°; cochromatography in five TLC systems [5], ^{1}H NMR, CIMS and EIMS.

Acknowledgements—Scholarship support to N.R.F. from the Consejo Desarrollo Cientifico y Humanistico, Universidad Central de Venezuela, and research funds from BMRG No. 507-RR-05586 (NIH) are gratefully acknowledged.

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Phytochemistry, Vol. 22, No. 9, pp. 2102–2104, 1983.
Printed in Great Britain.

0031–9422/83 $3.00 + 0.00

ANTIFUNGAL STRESS METABOLITES FROM *SOLANUM AVICULARE*

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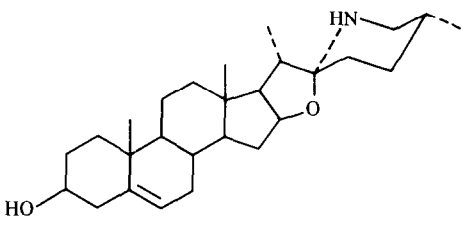
(*Revised received* 3 *February* 1983)

Key Word Index—*Solanum aviculare*; Solanaceae; solasodine; *Solanum* alkaloids; stress metabolites; vacuum infiltration.

Abstract—The steroidal alkaloids 5β-solasodan-3-one and solasodenone were isolated as antifungal stress metabolites from leaves of *Solanum aviculare* stressed by vacuum infiltration with water. The constitutive leaf alkaloids, solasodine and solamargine, also showed antifungal activity.

INTRODUCTION

Solanum aviculare L. has been used commercially as a source of 'solasonine', a mixture of steroidal glycoalkaloids from which solasodine (**1**), a useful starting material in steroidal drug synthesis, is obtained on hydrolysis [1]. The chemistry of the *Solanum* alkaloids has recently been reviewed [2]. Fungitoxicity for the steroidal alkaloids and glycoalkaloids of the Solanaceae has been reported for solasodine and solamargine from *S. aviculare* [3], solanine from the potato (*S. tuberosum*) [3] and tomatine from the tomato (*Lycopersicon esculentum* [3, 4]. Fungitoxic compounds may also accumulate in plants following fungal infection (phytoalexins) or stress induced by abiotic agents including detergents, metal salts, chloroform vapour, freeze–thaw treatment [5], UV radiation [6] or vacuum infiltration [7, 8]. In tomato and potato the antifungal sesquiterpene rishitin is synthesized under fungal challenge [9]. Here we report the isolation of the *Solanum* alkaloids 5β-solasodan-3-one and solasodenone as antifungal stress metabolites from the leaves of *S. aviculare* subjected to vacuum infiltration with water.



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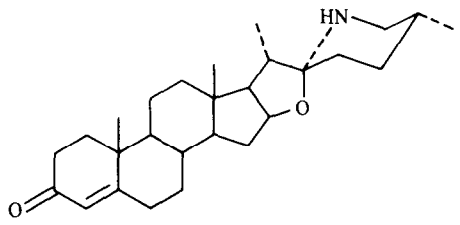

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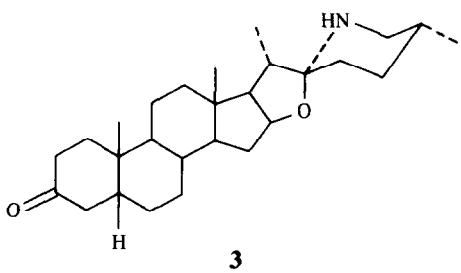

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RESULTS AND DISCUSSION

Antifungal bioassay monitored fractionation of the extracts from vacuum infiltrated leaves of *S. aviculare* led to the isolation of the *Solanum* alkaloids **1**–**3** together with the constitutive glycoalkaloid solamargine. Solamargine was purified by droplet counter current chromatography and identified from its ^{13}C NMR spectrum [10] and by its hydrolysis to solasodine, glucose and rhamnose. Solamargine was the only glycoalkaloid present which showed activity in our TLC bioassay system. From the less polar fractions solasodine (**1**) was readily identified by comparison with an authentic sample. Only trace amounts of solasodine are normally present in *S. aviculare* [1]. Alkaloids **2** and **3** were separated by careful chromatography and fractional crystallization. They were not found in untreated leaves nor in freshly harvested material. Solasodenone (**2**), the less polar of the two, was identified from its ^{13}C NMR data and by comparison with data published for material isolated from *S. hainanense* [11]. Solasodenone (**2**) has not previously been reported from *S. aviculare*, nor has its ^{13}C NMR been reported (see Experimental).

Alkaloid **3**, $C_{27}H_{43}NO_2$ (*m/z* 413.3304), showed major high resolution ion fragments at *m/z* 138 ($C_9H_{16}N$) and 114 (C_6H_2NO), characteristic of a spirominoketal system [2]. The IR spectrum indicated a saturated ketone (1700 cm^{-1}). Comparison of the ^{13}C NMR spectra of **3** with that of solasodine (**1**) [12] and with the spectra of appropriate steroidal model compounds [13] gave the structure as 5β-solasodan-3-one (**3**). The alternative 5α-configuration was readily eliminated from ^{13}C NMR evidence. 5α-Androstan-3-one shows a distinctive high field C-19 methyl (δ11.4) and a lower field C-5 methine (δ46.8) [13]. Neither resonance is observed for **3**, instead the observed methyl and methine resonances at δ22.7 and 44.2 respectively are in excellent agreement for those reported [13] for C-19 and C-5 of 5β-cholestan-3-one (δ22.7 and 44.4, respectively). 5β-Solasodan-3-one (**3**) has not previously been reported from natural sources but has been prepared by partial synthesis from solasodine (**1**) [2].

The insolubility in water of the steroidal alkaloids hindered quantitative bioassay. In a germ tube growth inhibition bioassay they proved only weakly active: solasodenone $ED_{50} > 100\ \mu g/ml$, 5β-solasodan-3-one ED_{50} 70 μg/ml and solasodine 35% inhibition at 25 μg/ml but with no increase in inhibition at higher concentrations. The alkaloids **1**–**3** were not detected as diffusates into aqueous spore suspension droplets [9] placed onto the leaves of *S. aviculare*. While this may reflect their insolubility in water, the comparably lipophilic bean phytoalexin phaseollin can be produced by both drop diffusate [9] and vacuum infiltration [7] techniques. All attempts to effect the synthesis of **2** and **3** in buffered leaf homogenates failed; only solasodine (**1**) being detected in most cases. Treatment with toluene vapour, used to disrupt cell membranes in assays for cyanogenesis [14], also failed to produce other than traces of solasodine (**1**). The biological role of alkaloids **1**–**3**, therefore, remains uncertain and further work will be required to define their role in the disease resistance of *S. aviculare*.

EXPERIMENTAL

Mp, uncorr.; ^1H NMR (79.54 MHz) and ^{13}C NMR (20 MHz); $CDCl_3$, TMS as int. standard. ^{13}C multiplicities were determined using the grated-decoupling spin echo sequence GASPE [15] to give methyl and methine resonances as inverted singlets. IR, UV and α_D: $CHCl_3$ unless otherwise stated; TLC: sprayed with vanillin for the alkaloids and H_2SO_4–95% EtOH for the sugars. Alkaloids **1**, **2** and **3** gave distinct colour reactions on slow heating with vanillin (brown → blue black, orange → brown and yellow → deep brown, respectively).

TLC bioassay. Fractions were chromatographed on Si gel plates, then sprayed with a suspension of *Cladosporium cladosporioïdes* conidia in a nutrient soln [16]. The detection limit for solasodine was 1 μg. Duplicate plates were sprayed with vanillin.

Germ tube growth inhibition bioassay. Sporelings (17 hr old) of *Colletrotrichum gloeosporïodes*, supported on 4 mm diameter cellulose discs and grown on potato dextrose agar, were placed in 0.1 ml droplets of a sucrose–casein hydrolysate (acid) medium containing the antifungal compound [17]. DMSO, final concn 2%, was required to solubilize the alkaloids. Growth measurements, as a function of concn were made after 6 hr incubation at 20°.

Isolation of alkaloids. Branches of *S. aviculare* were collected from cropped fields grown at Waitere, New Zealand. After storage overnight at 4° the leaves were removed and cut into 4–6 cm lengths. They were floated in H_2O in a Buchner flask and subjected to vacuum at a water pump for 1 min using gentle swirling to dislodge air bubbles. On release of the vacuum, the now darkened leaves sank. After draining off excess H_2O, the leaves were laid out on damp paper and incubated in covered trays at 25° for 3 days. Control leaves were incubated but not subjected to vacuum infiltration.

After 3 days, the leaves were washed and shaken with 95%

EtOH. Control and fresh leaf material was extracted in a Waring blender. The extract was filtered and adjusted to a nominal '80% EtOH' by the addition of H_2O. Partitioning with petrol gave the bulk of the antifungal activity in this phase. Partition of the remaining aq. phase with $CHCl_3$–EtOH–H_2O (1:1:1) gave further active material in the $CHCl_3$ phase (solamargine and minor quantities of **1**–**3**).

Active fractions were separated from the petrol and $CHCl_3$–EtOH extracts by Si gel chromatography using EtOAc–petrol or MeOH–EtOAc, respectively. For the controls the combined petrol and $CHCl_3$–EtOH phases were eluted from Si gel with MeOH–$CHCl_3$ (1:9). Further purification was accomplished on Si gel (MeOH–$CHCl_3$, 1:9; Me_2CO–$CHCl_3$, 1:4), Sephadex LH-20 (petrol–$CHCl_3$–EtOH, 10:10:1) and/or Florisil (Me_2CO–$CHCl_3$, 1:4) to a stage where crystallization from Me_2CO–H_2O allowed separation from the accompanying pigments. Alkaloids were desalted by extraction into NH_3–$CHCl_3$.

Solamargine. $[\alpha]_D^{25}$ −105° (EtOH; *c* 2.2), lit. −106° (EtOH) [10] was isolated directly from a crude Si gel column fraction by droplet counter current chromatography [Tokyo Rikakikai, DCC-A, $CHCl_3$–MeOH–H_2O (7:13:8), ascending mode]. Acid hydrolysis with 2 N CF_3COOH (3 hr, 80°) gave solasodine, glucose and rhamnose by TLC (MeOH–$CHCl_3$, 1:9 and Me_2CO–$CHCl_3$–MeOH–H_2O, 15:2:2:1).

Solasodine (**1**). Mp 199°–201° was identified by direct comparison (TLC, IR, MS and ^1H NMR) with an authentic sample.

Solasodenone (**2**). Needles from Me_2CO–H_2O, mp 177–179°, lit. [11] mp 178°. ^{13}C NMR: δ15.4 (C-21), 16.6 (C-18), 17.5 (C-19), 19.4 (C-27), 21.0 (C-11), 30.3 (C-24), 31.3 (C-25), 32.2 (C-7), 32.3 (C-15), 32.9 (C-6), 34.1 (C-2), 34.1 (C-23), 35.3 (C-8), 35.8 (C-1), 38.8 (C-10), 39.9 (C-12), 40.8 (C-13), 41.4 (C-20), 47.7 (C-26), 53.9 (C-9), 55.8 (C-14), 62.9 (C-17), 79.0 (C-16), 98.4 (C-22), 124.0 (C-4), 171.0 (C-5), 199.3 (C-3).

5β-Solasodan-3-one (**3**). Needles from Me_2CO–H_2O, mp 169–170°, lit. [2] mp 164–165°; $[\alpha]_D^{20}$ −49° (*c* 2.8); IR ν_{max} cm^{-1}: 1700, 1140, 1087, 970, 965 and 876: ^1H NMR: δ0.82 (3H, *s*, H-18), 0.83 (3H, *d*, *J* = 6 Hz, H-27), 0.94 (3H, *d*, *J* = 6 Hz, H-21), 1.04 (3H, *s*, H-19), 2.63 (2H, *m*, H-26), 4.26 (1H, *m*, H-16α); ^{13}C NMR: δ15.3 (C-21), 16.6 (C-18), 19.3 (C-27), 21.1 (C-11), 22.7 (C-19), 26.1 (C-6), 26.6 (C-7), 30.4 (C-24), 31.4 (C-25), 32.1 (C-15), 34.1 (C-23), 35.0 (C-10), 35.2 (C-8), 37.0 (C-2), 37.2 (C-1), 40.3 (C-12), 40.9 (C-13), 40.9 (C-9), 41.3 (C-20), 42.3 (C-4), 44.2 (C-5), 47.7 (C-26), 56.3 (C-14), 63.1 (C-17), 78.7 (C-16), 98.2 (C-22), 212.9 (C-3), EIMS *m/z* (rel. int.): 413.3304 (9) ($C_{27}H_{43}NO_2$ requires 413.3294), 138.1274 (71) and 114.0921 (100).

Acknowledgements—We gratefully thank Solexin Ltd, Waitere, for providing plant material, Dr D. R. Biggs, DSIR, Palmerston North, for helpful suggestions, Professor R. Hodges, Massey University, for high resolution mass spectra, and Dr R. H. Newman, DSIR, Petone, for running the NMR spectra.

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