Bioactive Steroidal Alkaloids from *Solanum umbelliferum*

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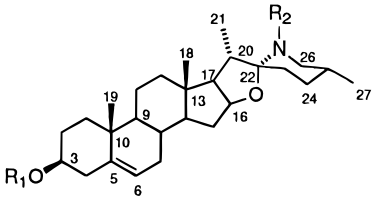
Bioassay-directed fractionation of the MeOH extract of *Solanum umbelliferum* afforded solasodine (**1**), *O*-acetylsolasodine (**2**), and solasodine 3-*O*-*β*-D-glucopyranoside (**3**). Alkaloids **1** and **2** exhibited significant activity toward DNA repair-deficient yeast mutants, whereas **3** and the synthetic analogues *N*-acetylsolasodine (**4**) and *N*,*O*-diacetylsolasodine (**5**) were found to be inactive. Compounds **2** and **3** are new natural products.

Steroidal alkaloids and their glycosides occurring in numerous species of *Solanum* are known to possess a variety of biological activities including antifungal,[1−3] antiviral,[4] molluscicidal,[5] teratogenic, and embryotoxic.[6] Preparations containing solasodine glycosides are currently being employed for the treatment of certain skin cancers.[7]

In the course of our random screening of plants for potential anticancer activity utilizing a mechanism-based yeast bioassay[8,9] a MeOH extract of *Solanum umbelliferum* Eschs. (Solanaceae) was shown to exhibit promising activity. Bioassay-guided fractionation afforded solasodine (**1**), *O*-acetylsolasodine (**2**), and solasodine 3-*O*-*β*-D-glucopyranoside (**3**). In a preliminary structure−activity relationship study, *N*-acetylsolasodine (**4**) and *N*,*O*-diacetylsolasodine (**5**) were synthesized starting from solasodine (**1**) and subjected to our mechanism-based yeast bioassay along with the natural alkaloids **1**−**3**. In this paper, we report the structure elucidation of the new alkaloids **2** and **3**, synthesis of **4** and **5**, and biological evaluation of **1**−**5**.



1 $R_1 = R_2 = H$

2 $R_1 = Ac$, $R_2 = H$

3 R_1 = 6′-OH, 5′-O, 1′-HO, OH, OH; $R_2 = H$

4 $R_1 = H$, $R_2 = Ac$

5 $R_1 = R_2 = Ac$

6 $R_1 = OSiEt_3$, $R_2 = H$

7 $R_1 = OSiEt_3$, $R_2 = Ac$

The whole plant of *S. umbelliferum* was sequentially extracted with hexane, MeCOEt, and MeOH. Bioassay-guided fractionation of the bioactive MeOH extract involving solvent−solvent partitioning and gel-permeation, Si gel, and RP chromatography afforded the two active compounds **1** and **2** and the inactive compound **3**. All three compounds showed a positive response to an alkaloid test on TLC and exhibited some common features in their ^{1}H-NMR spectra. Comparison of physical and spectral data of **1** with those of various *Solanum* alkaloids suggested it to be solasodine, a steroidal alkaloid common to many *Solanum* species.[10]

On the basis of ^{1}H-NMR spectral data compound **2** was an acetyl derivative of **1**, and this was confirmed by comparison of its mp, $[\alpha]_D$, ^{13}C-NMR, and MS data with those reported for *O*-acetylsolasodine.[10] Compound **3** was obtained as a white amorphous powder, mp 246−248 °C, $[\alpha]_D$ −81.1°. FABMS and ^{1}H- and ^{13}C-NMR spectral data indicated it to be a glucosyl derivative of solasodine. Physical data (mp, $[\alpha]_D$) of this compound compared well with those reported for solasodine 3-*O*-*β*-D-glucopyranoside (**3**).[11] A literature search revealed the absence of any ^{13}C-NMR spectral data for **3**. Therefore, a ^{13}C-NMR study was undertaken, and the assignments were made by comparison with ^{13}C-NMR spectral data reported for related compounds.[12] Although compounds **2** and **3** have been prepared from solasodine and solamargine, respectively,[10,11] they are both new natural products.

Compounds **1**−**3** were tested for their potential anticancer activity utilizing our mechanism-based bioassay employing genetically engineered yeast mutants.[9,13] Solasodine (**1**) and *O*-acetylsolasodine (**2**) showed significant activity in this bioassay, whereas solasodine 3-*O*-*β*-D-glucopyranoside (**3**) was found to be inactive even at a dose of 8000 *μ*g/mL (Table 1). The inactivity of **3** may be attributed to its inability to permeate through the yeast cell wall.

It was of interest to determine the functional moiety in **1** and **2** responsible for their DNA-damaging activity. On the basis of our previous studies on the biological activity of 3*β*-hydroxyergost-5-ene derivatives,[9] we hypothesized that the DNA-modifying activity of **1** and **2** may be due to the spiro-aminoacetal function present in the steroidal side chain. This spiro-aminoacetal group may open up, producing an electrophilic iminium species capable of alkylating DNA (Scheme 1). To test this hypothesis, *N*-acetyl- (**4**) and *N*,*O*-diacetylsolasodine (**5**) were prepared, since the lone pair of electrons on N is unavailable to form the putative bioactive iminium ion intermediate in these compounds. Direct acetylation of **1** with excess Ac_2O/pyridine gave *N*,*O*-diacetylsolasodine (**5**). Preparation of *N*-acetylsolasodine (**4**) in-

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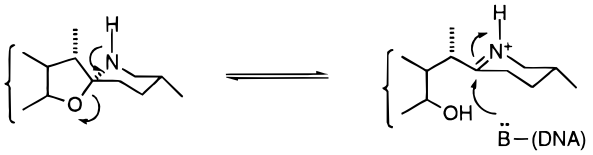
Table 1. Bioactivity of Steroidal Alkaloids **1**−**3** and their Derivatives **4** and **5**[a]

compd	yeast strain			
	RS 322 YK (rad 52Y)	RS 321	RS 167 N (rad 6)	RS 188 N (RAD^+)
1	5.0	1.9	>10.0	6.1
2	21.0	3.0	30.0	15.7
3	NA[b]	NA[b]	NA[b]	NA[b]
4	NA[b]	NA[b]	NA[b]	NA[b]
5	NA[b]	NA[b]	NA[b]	NA[b]
camptothecin[c]	0.9			100

[a] Results are expressed as IC_{12} values in μg/mL (concentration required to produce an inhibition zone of 12 mm around a 100 μL well in the yeast strain). [b] NA = not active at a dose of 8000 μg/mL. [c] Standard reference compound.

Scheme 1. Proposed Mechanism of DNA-Damaging Activity of Steroidal Alkaloids



volved the protection of the 3β-hydroxy function of solasodine as its triethylsilyl ether (**6**), acetylation (AcCl/CH_2Cl_2/Et_3N) to obtain **7**, followed by deprotection of the silyl ether with HF/pyridine.

When subjected to our DNA-modifying bioassay, both derivatives were found to be inactive, supporting the possible participation of the spiro-aminoacetal function in DNA-modifying activity of these steroidal alkaloids.

Experimental Section

General Experimental Procedures. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Optical rotaions were taken with a Perkin-Elmer Model 241 polarimeter. The ^1H and ^{13}C NMR spectra were recorded on a Varian Unity 400 spectrometer at 400 and 100.57 MHz, respectively, with TMS as internal standard. $^1H-^1H$ COSY and DEPT NMR experiments were performed on the same spectrometer, using standard Varian pulse sequences. Flash chromatography were performed using Si gel Merck G60 (230−400 mesh), Sorbsil RP-18 (Phase Separations Ltd), and RP preparative TLC with Whatman PLKC 18F linear-K RP (250 μm, 20 × 20 cm) plates. Sephadex LH-20 (Sigma) was employed for gel permeation chromatography.

Plant Material. *S. umbelliferum* (B 633394) was collected in California in March 1965 (acquisition no. E 1104) and was subsequently transferred to Biotics Ltd., University of Sussex, Brighton, U.K. A voucher specimen is located at the herbarium of the U. S. National Arboretum, Washington, DC.

Bioassays. The procedures for mechanism-based yeast bioassays have been described elsewhere.[9] The IC_{12} values refer to the concentration in μg/mL required to produce a zone of inhibition of 12 mm diameter around a 100 μL well during a 48-h incubation period at 37 °C.

Extraction and Isolation. *S. umbelliferum* (whole plant; 600 g) was extracted sequentially with hexane, MeCOEt, and MeOH. The bioactive MeOH extract (10 g) [IC_{12} (RS 321) 2500 μg/mL] was partitioned between hexane and 80% aqueous MeOH. H_2O was added to the aqueous MeOH fraction until a 60% aqueous MeOH mixture was produced. This was extracted thoroughly with $CHCl_3$. The $CHCl_3$ extract was dried under reduced pressure to yield 1.3 g of a bioactive fraction. This was subjected to gel permeation chromatography on Sephadex LH-20 eluting initially with hexane−CH_2Cl_2 (4:1), followed by hexane−CH_2Cl_2 (1:1), CH_2Cl_2, and finally MeOH. The bioactive hexane−CH_2Cl_2 (4:1) fraction (108 mg) was further subjected to flash chromatography on Si gel and eluted with $CHCl_3$−MeOH−H_2O (9.0:1.0:0.1). On the basis of their TLC patterns, similar fractions were combined to obtain a total of four fractions. Fraction 2 (45 mg) was purified by Si gel column chromatography (CC) (eluent:EtOAc−hexane, 9:1) followed by reversed-phase CC and eluted with 90% aqueous MeOH to yield *O*-acetylsolasodine (**2**) (2.1 mg). The hexane−CH_2Cl_2 (1:1) fraction (147 mg) resulting from gel-permeation CC was subjected to flash chromatography on Si gel and eluted with $CHCl_3$−MeOH−H_2O (9.0:1.0:0.1) to obtain a total of four fractions. Fraction 3 (85 mg) from this column was purified by Si gel CC (eluent: EtOAc) to afford solasodine (**1**) (60 mg). The CH_2Cl_2 fraction (160 mg) obtained from the above Sephadex column was loaded onto a Si gel column and eluted with $CHCl_3$−MeOH−H_2O (4.0:1.0:0.1) to obtain a total of five fractions. Fraction 4 (40 mg) was purified by reversed-phase preparative TLC (MeOH−H_2O, 9:1) to yield solasodine 3-*O*-β-D-glucopyranoside (**3**) (14 mg).

Solasodine (**1**): colorless crystals (MeOH); mp 198−200 °C (lit.[10] mp 200−202 °C); $[\alpha]^{26}_D$ −108.4° (*c* 0.62, $CHCl_3$) [lit.[10] $[\alpha]^{26}_D$ −116.7° ($CHCl_3$)]. Identified by comparison of MS and ^1H and ^{13}C NMR data with those reported.[10]

***O*-Acetylsolasodine** (**2**): white amorphous powder; mp 192−194 °C [lit.[10] mp 191−193 °C]; $[\alpha]^{26}_D$ −95.5° (*c* 0.20, $CHCl_3$) [lit.[10] $[\alpha]^{26}_D$ −109.2° ($CHCl_3$)]. Identified by comparison of MS and ^1H and ^{13}C NMR data with those reported.[10]

Solasodine 3-*O*-β-D-glucopyranoside (3): white amorphous powder; mp 246−248 °C [lit.[11] mp 256−259 °C]; $[\alpha]^{26}_D$ −81.1° (*c* 0.24, MeOH) [lit.[11] $[\alpha]^{26}_D$ −87° (MeOH)]; FABMS (positive mode) *m/z* $[M + H]^+$ 576 (23); ^1H NMR (CD_3OD, 400 MHz) δ 0.83 (3 H, s, Me-18), 0.85 (3 H, d, J = 6.2 Hz, Me-27), 0.97 (3 H, d, J = 7.2 Hz, Me-21), 1.04 (3 H, s, Me-19), 2.57 (2 H, m, H-26), 3.58 (1 H, m, H-3), 4.34 (1 H, m, H-16), 4.37 (1 H, d, J = 7.8 Hz, H-1′), 5.37 (1 H, br d, J = 5.3 Hz, H-6); ^{13}C NMR (CD_3OD, 100 MHz) δ 142.0 (s, C-5), 122.5 (d, C-6), 102.4 (d, C-1′), 99.4 (s, C-22), 80.5 (d, C-3), 79.7 (d, C-16), 78.0 (d, C-3′[a]), 77.8 (d, C-5′[a]), 75.1 (d, C-2′), 71.6 (d, C-4′), 63.9 (d, C-17), 62.7 (t, C-6′), 57.7 (d, C-14), 51.7 (d, C-9), 48.2 (t, C-26), 42.7 (d, C-20), 41.6 (s, C-13), 40.9 (t, C-4), 39.7 (t, C-12), 38.5 (t, C-1), 38.0 (s, C-10), 34.8 (t, C-23), 33.2 (t, C-7), 33.0 (t, C-15), 32.7 (d, C-8), 31.5 (d, C-25), 31.0 (t, C-2[b]), 30.7 (t, C-24[b]), 22.0 (t, C-11), 19.8 (q, C-19[c]), 19.7 (q, C-27[c]), 16.8 (q, C-18), 15.4 (q, C-21).[14]

***N*-Acetylsolasodine** (**4**). To a stirred solution of solasodine (**1**) (8.0 mg) in dry DMF (0.2 mL) was added chlorotriethylsilane (6.0 mg) followed by imidazole (3.0 mg). After 3 h of stirring at 25 °C (TLC control), the reaction mixture was worked up in the usual manner, giving the crude product which on purification by PTLC (SiO_2; $CHCl_3$−MeOH, 9:1) afforded *O*-(triethylsilyl)-solasodine (**6**) as an amorphous white solid (10.5 mg):

MS *m/z* [M^+] 527 (100), [$M^+ - Me$] 512 (15), [$M^+ - 28$] 499 (95), 484 (17), 480 (13), 428 (11), 394 (14), 388 (47), 348 (15), 317 (22), 282 (51), 267 (30), 253 (51), 228 (28), 213 (38); ^{1}H-NMR ($CDCl_3$) δ 0.57 (6 H, q, $J = 7.9$ Hz, 3 $\times$ $SiCH_2CH_3$), 0.78 (3 H, s, Me-18), 0.82 (3 H, d, $J = 6.3$ Hz, Me-27), 0.93 (9 H, t, $J = 7.9$ Hz, 3 x $SiCH_2CH_3$), 0.98 (3 H, d, $J = 6.6$ Hz, Me-21), 0.99 (3 H, s, Me-19), 2.60 (2 H, m, H-26), 3.45 (1 H, m, H-3), 4.27 (1 H, m, 16α-H), 5.37 (1 H, br d, $J = 5.4$ Hz, H-6); ^{13}C-NMR ($CDCl_3$) δ 141.6 (s, C-5), 120.9 (d, C-6), 98.2 (s, C-22), 78.8 (d, C-16), 72.3 (d, C-3), 62.8 (d, C-17), 56.6 (d, C-14), 50.2 (d, C-9), 47.7 (t, C-26), 42.8 (t, C-4), 41.2 (d, C-20), 40.5 (s, C-13), 40.0 (t, C-12), 37.4 (t, C-1), 36.7 (s, C-10), 34.0 (t, C-23), 32.2 (t, C-2), 32.1 (t, C-7, C-15), 31.4 (d, C-8, C-25), 30.3 (t, C-24), 20.9 (t, C-11), 19.4 (q, C-27), 19.3 (q, C-19), 16.4 (q, C-18), 15.3 (q, C-21), 6.9 (q, $SiCH_2CH_3$), 4.9 (t, $SiCH_2$).

O-(Triethylsilyl)solasodine (**6**) (5.0 mg) was acetylated with AcCl (0.1 mL) in dry CH_2Cl_2 (0.25 mL) containing Et_3N (0.1 mL) and (*N*,*N*-dimethylamino)pyridine (1.0 mL) at 0 °C. After 1 h, the cooling bath was removed and the reaction mixture stirred for 2 h at 25 °C. Usual workup afforded *N*-acetyl-*O*-(triethylsilyl)solasodine (**7**) as a colorless thick gum (4.0 mg) which resisted crystallization: MS *m/z* [M^+] 569 (38), [$M^+ - Me$] 554 (70), 540 (27), 526 (20), 500 (27), 469 (21), 385 (85), 283 (67), 253 (100), 214 (48); ^{1}H NMR ($CDCl_3$) δ 0.60 (6 H, q, $J = 8.0$ Hz, 3 $\times$ $SiCH_2CH_3$), 0.80 (3 H, s, Me-18), 0.82 (3 H, d, $J = 6.0$ Hz, Me-27), 0.90 (9 H, t, $J = 8.0$ Hz, 3 $\times$ $SiCH_2CH_3$), 1.00 (3 H, s, Me-19), 1.10 (3 H, d, $J = 6.5$ Hz, Me-21), 2.20 (3 H, s, $NCOCH_3$), 2.85 and 3.15 (1 H each, m, CH_2-26), 3.45 (1 H, m, H-3), 4.00 (1 H, m, H-25), 4.27 (1 H, m, 16α-H), 5.30 (1 H, br s, H-6).

To a stirred solution of **7** (3.0 mg) in freshly distilled dry THF (0.2 mL) was added HF/pyridine (10 μL). After being stirred for 2.5 h at 25 °C (TLC control), the reaction mixture was worked up in the usual manner to afford a pale yellow semisolid (2.8 mg) which was purified by PTLC (SiO_2; $CHCl_3$−MeOH−H_2O, 9.0:1.0:0.1) to afford *N*-acetylsolasodine (**4**) as a colorless amorphous solid (1.95 mg) having ^{1}H NMR and MS data identical with those reported for this derivative.[10]

***N*,*O*-Diacetylsolasodine** (**5**). Acetylation of solasodine (**1**) (10.0 mg) with Ac_2O (20 μL) in dry pyridine (0.2 mL) for 7 h at 25 °C followed by usual workup and purification by Si gel CC (eluent: $CHCl_3$−MeOH−H_2O, 9.0:1.0:0.1) afforded *N*,*O*-diacetylsolasodine (**5**) as a colorless powder (8.0 mg): mp 160−161 °C; $[\alpha]^{26}_D$ −49.2° (*c* 0.20, $CHCl_3$) [lit.[10] mp 164−165 °C; $[\alpha]_D$ −54.2° ($CHCl_3$)]. Identified by comparison of MS, and ^{1}H and ^{13}C NMR data with those reported.[10]

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(14) Signals bearing the same superscript may be interchanged.

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