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PHYTOCHEMISTRY
Phytochemistry 65 (2004) 1283–1286
www.elsevier.com/locate/phytochem

Arudonine, an allelopathic steroidal glycoalkaloid from the root bark of *Solanum arundo* Mattei

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Received in revised form 3 December 2004
Available online 6 May 2004

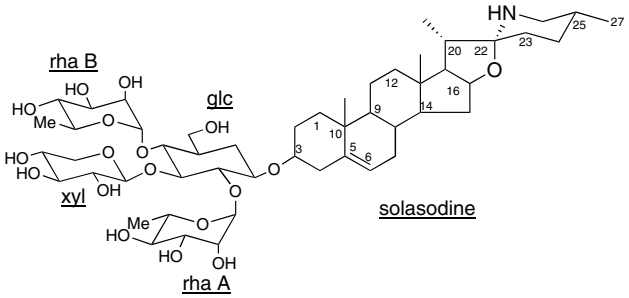
Abstract

Bioassay-guided fractionation of the methanol extract of the root bark of *Solanum arundo* Mattei led to the isolation of a steroidal glycoalkaloid, designated arudonine, which was elucidated to be solasodine *O*-α-L-rhamnopyranosyl-(1→2)-{[β-D-xylopyranosyl-(1→3)], [α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside. This steroidal glycoalkaloid inhibited the growth of lettuce seedlings (*Lactuca sativa*).

Keywords: *Solanum arundo*; Solanaceae; Steroidal glycoalkaloid; Arudonine; Allelopathic effect; Plant growth inhibitory activity

1. Introduction

Several species of *Solanum* in East Africa are known to be poisonous and are reportedly used to induce miscarriages (Verdcourt and Trump, 1969). During our plant collection trips to various parts of Kenya, we also observed that other plants were rarely found in the proximity of *S. arundo* Mattei (Solanaceae); this may be due to an allelopathic effect. In our preliminary assays, the crude methanol extract of the root bark of *S. arundo* was observed to inhibit the growth of lettuce (*Lactuca sativa*) seedlings at 3000 ppm. Bioassay-guided fractionation indicated that the active principle remained in the aqueous layer after extraction with organic solvents by partitioning. According to a TLC analysis, the bioactive fraction was positive toward Dragendorff's reagent, which suggested the presence of alkaloids, and more specifically steroidal glycoalkaloids. Steroidal alkaloids are mainly found in the genus *Solanum* (Ripperger and Schreiber, 1981; Ripperger, 1998) and are of interest with regard to both ecology and human health. In the wild, they are thought to be an important component of the plant's chemical armory against herbivores and microbial pathogens (Roddick, 1996). Previously, the two allelopathic alkaloids solamargine and solasonine were isolated from the ripe fruits of *S. incanum* by a combination of two countercurrent chromatographic steps: rotation locular countercurrent chromatography (RLCC) and droplet countercurrent chromatography (DCCC) (Fukuhara and Kubo, 1991). This paper presents the structure elucidation of a new steroidal glycoalkaloid, designated arudonine (**1**), obtained by bioassay-guided fractionation.



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doi:10.1016/j.phytochem.2004.03.025

2. Results and discussion

2.1. Identification of steroidal glycoalkaloid (1)

Bioassay-guided fractionation of the methanol extract of the root bark of *S. arundo* led to the isolation of **1**. Compound **1** had a chemical formula of $C_{50}H_{80}O_{19}N$ by positive-ion HR-FABMS (m/z 1000.3454 calc. for $C_{50}H_{81}O_{19}N$, 1000.3454). The ^1H and ^{13}C-NMR spectral data, based on a comparison with values in the literature for the two known steroidal glycoalkaloids solamargine and solasonine (Fukuhara and Kubo, 1991), suggested that **1** should consist of a solasodine skeleton as the aglycone and four sugar moieties, including one hexose, one pentose and two rhamnose moieties. An analysis by collision activated dissociation (CAD) with argon as the target gas provided many clues regarding the sequences of the sugar moieties. The CAD mass spectrum against $[M-H]^-$ (negative FABMS) at m/z 998 gave several fragments at m/z 886 ($[M-H-132]^-$) and m/z 852 ($[M-H-146]^-$), which corresponded to the loss of a pentose and each rhamnose. The peaks at m/z 720 ($[M-H-132-146]^-$) and m/z 574 ($[M-H-132-146-146]^-$) corresponded to the loss of a pentose and rhamnose, and a pentose and two rhamnose moieties, respectively. In the CAD spectrum at m/z 1000 using positive FAB-MS, several peaks were observed at m/z 982, 850, 836, 704, and 576, which corresponded to $[M+H-18]^+$, $[M+H-18-132]^+$, $[M+H-18-146]^+$, $[M+H-18-132-146]^+$, and $[M+H-18-132-146-146]^+$, respectively. The fragment at m/z 414 ($[\text{solasodine}+H]^+$) corresponded to further losses of a pentose (132), two rhamnose (146 + 146) and hexose (162) moieties from m/z 1000, respectively. Based on these CAD results, the pentose and two rhamnose moieties should be attached by glycosidic linkages to hexose, which is attached to the 3-position of the solasodine moiety. The assignment of all protons of the sugar moieties in **1** was clarified by ^1H–^1H COSY and a selective 1D-HOHAHA experiment. For the selective 1D-HOHAHA experiment and the spin–spin connectivities based on ^1H–^1H COSY spectra, selective excitation of the anomeric proton (hexose) at δ 4.86 (d, $J = 7.4$ Hz) gave six peaks, and the proton signals at δ 4.44 (*bd*, 3,4-H), 4.10 (*m*, 6-H), 4.05 (*m*, 2-H), 4.0 (*m*, 6-H) and 3.6 (*m*, 5-H) were assigned. Excitation at δ 5.23 (anomeric-H of pentose, d, $J = 7.4$ Hz) gave five signals at δ 4.46 (*m*, 4-H), 4.28 (*m*, 5-H), 4.12 (*bdd*, 2-H), 4.08 (*bdd*, 3-H) and 3.54 (*m*, 5-H). The higher-field broad singlet anomeric proton (δ 5.84) in rhamnose connected to five protons at 5.08 (*dd*, $J = 6.1$, 9.1 Hz), 4.62 (*bd*, 3-H), 4.58 (*bs*, 2-H), 4.16 (*bt*, $J = 9.1$ Hz, 4-H) and 1.41 (d, $J = 6.1$ Hz, 6-CH_3). Selective excitation of the 6-methyl proton at δ 1.65 (d, $J = 6.1$ Hz) in rhamnose gave five proton signals at δ 6.1 (*bs*, anomeric-H), 4.90 (*bd*, 2-H), 4.73 (*dd*, $J = 6.1$, 9.1 Hz, 5-H), 4.44 (*bd*, 3-H) and 4.22 (*bt*, $J = 9.1$, 4-H). All of the carbon signals from the sugar moieties of **1** were assigned based on ^1H–^{13}C COSY spectra. In the ^{13}C chemical shift values of pentose and rhamnose, no glycosyl downfield shifts were observed except for those of three anomeric carbons at δ104.70 (pentose), 102.65 (rhamnose A), and 101.65 (rhamnose B) (Tanaka, 1985). These results and additional CAD data indicated that each of these three sugar residues should be attached to hexose by a glycosidic linkage. The structure of the pentose was determined to be β-D-xylose based on signals at δ 67.32 (*t*, xyl-5) and 104.70 (*d*, xyl-1), and the J values (JC1–H1 = 152 Hz and J1H–2H = 7.4 Hz) (Tanaka, 1985; Kubo and Kim, 1987). The anomeric configurations of the two rhamnose moieties were also deduced from the anomeric δ and J values, and were determined to be α-L-rhamnose based on δ 102.65 (*d*, rha A-1), δ 101.65 (*d*, rha B-1) and JC1–H1 = 165–166 Hz (Tanaka, 1985). On the other hand, the hexose showed downfield shifts for C2, C3 and C4, which suggest that it is either β-D-glucose or β-D-galactose (JC1–H1 = 154 Hz and J1H–2H = 7.4 Hz). The structure of the hexose was determined to be as shown in Fig. 1 by a NOESY experiment. Cross-peaks in the NOESY spectrum were observed between the hexose anomeric proton signal at δ 4.86 and both the 3-axial proton signal at δ 4.44 and the 5-axial proton signal at δ 3.60. Furthermore, the 2-axial proton signal at δ 4.05 was related to the 4-axial proton signal at δ 4.44 by the presence of cross-peaks. These results indicated that the hexose should be β-D-glucoserather than β-D-galactose. The NOESY spectrum also provided evidence of a glycosyl linkage between glucose and the 3-β position of the solasodine moiety. The points of linkage between glucose and the xylose, rhamnose A and rhamnose B residues were determined by NOESY and HMBC analysis. In the NOESY experiment (Fig. 1), the anomeric proton signal of rhamnose A at δ 6.1 was related not only to the 4-axial proton signal of glucose, but also to the 6-methylene proton signals, which supported the close

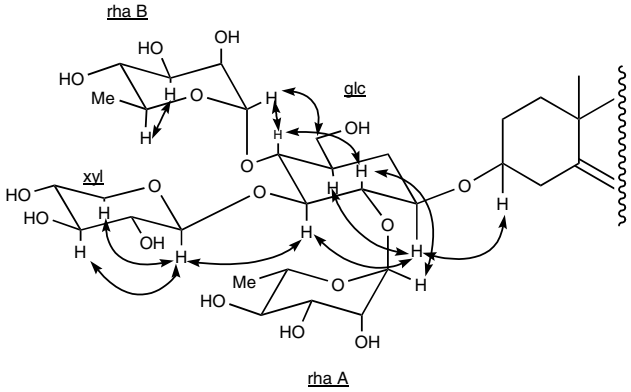

Fig. 1. NOESY correlations.

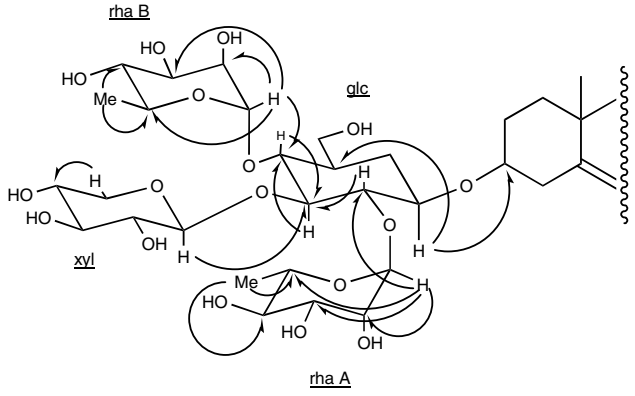

Fig. 2. HMBC correlations.

spatial orientation of the anomeric proton of rhamnose A and the primary alcohol part of glucose. The rhamnose B proton at δ 5.84 was related to the 2-axial proton of glucose. The xylose anomeric proton signal at δ 5.23 was related to the 3-axial proton signal of glucose, and the 3-axial proton and 5-axial methylene proton signals of xylose itself. In addition, numerous long-range connectivities were observed in the HMBC spectrum of the sugar moieties (Fig. 2). The anomeric proton of rhamnose A was linked to the glucose 4-carbon at δ 73.38 via a three-bond correlation, and also to the 2-, 3- and 5-carbons of rhamnose A. In rhamnose B and xylose, correlation between these anomeric protons and the 2-carbon (glc-2) at δ 79.51 and the 3-carbon (glc-3) at δ 80.68 across the glucose ethereal oxygen was observed in the HMBC spectrum. Thus, the sugar moieties of **1** have a unique structure consisting of glucose with three sugar residues (xylose and two rhamnose moieties). All of the spectral data suggested that the structure of **1** was solasodine *O*-α-L-rhamnopyranosyl-(1 → 2)-{[β-D-xylopyranosyl-(1 → 3)], [α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranoside.

2.2. Effect of arudonine (*1*) on plant growth

Based on the above-mentioned observation of a possible allelopathic effect for *S. arundo* in the East African savanna, the effect of the crude methanol extract of *S. arundo* (root bark) on plant growth was examined. As expected, this extract completely inhibited the growth of lettuce seedlings at 3000 ppm. The growth inhibitory activity of each portion was examined after partitioning between aqueous solution and organic solvents; *n*-hexane, chloroform and ethyl acetate, in this order. The biological activity resided in the aqueous portion. Further fractionation of the aqueous portion using countercurrent chromatographic methods, and SiO_2 and ODS chromatography, gave arudonine (**1**) as the active principle. The roots of lettuce seedling were completely inhibited by **1** at 1000 ppm. The inhibited lettuce roots lacked hairs and were brown in color. These observations were similar to those with two alkaloids, solamargine and solasonine, isolated from the methanol extract of *S. incanum* (Fukuhara and Kubo, 1991). *Solanum* plants have been reported to contain many highly polar steroidal glycoalkaloids, which might play a role in defense against other plants or microorganisms, as in the tomato (*Lycopersicon esculentum*), which contains the well-known alkaloid α-tomatine (Roddick, 1975). It should be noted that the inhibitory activity of solasodine was less than 30% at 1000 ppm in the lettuce seedling assay. Therefore, the sugar moiety of arudonine should play an important role in its growth inhibitory activity. Although we did not examine how much of these alkaloids are released from *S. arundo* and accumulate in the soil, steroidal glycoalkaloids such as arudonine (**1**) may play an important role as allelopathic chemicals.

3. Experimental

3.1. General

IR spectra were measured on a Perkin–Elmer 1310 IR Spectrometer. UV spectra were recorded by a Hitachi 100-80 spectrometer. ^{1}H and ^{13}C-NMR data were obtained by a JEOL GX-500 spectrometer, and 2D-NMR and the selective 1D-HOHAHA experiment in inverse mode were performed using Brucker AMX-300 and a JEOL α-500 spectrometers. The HRFABMS spectrum on a glycerol matrix was recorded with a JEOL SXYL02 spectrometer. The CAD experiment using argon gas on a glycerol matrix was performed using a Finnigan Mat TSQ-700 spectrometer. HPLC was performed on a model LP-1000 liquid chromatograph (Tokyo Rikakikai Co., Ltd.) equipped with a UV detector (JASCO model 870) and a Capcell Pack ODS column (5 m, 4 mm i.d. × 25 cm, Shiseido Co., Ltd.). RLCC was performed on an RLCC-A (Tokyo Rikakikai, Co., Ltd.).

3.2. Preparation of plant material

The root bark of *S. arundo* was collected near Nakuru, Kenya. The plant was identified by Geoffrey M. Mungai of the East African Herbarium, Nairobi, Kenya, where a voucher specimen was deposited. The air-dried sample was extracted with MeOH at room temperature, and the extract was evaporated in vacuo to afford a brown tar. The MeOH extract (15.3g) was partitioned into *n*-hexane (0.8 g)-, $CHCl_3$ (0.4 g)-, EtOAc (0.38 g)- and H_2O (12.2 g)-soluble portions. The H_2O fraction (7 g) was dissolved in 30 ml of H_2O and subjected to RLCC. RLCC was performed with H_2O as a stationary phase and a gradient mobile phase. An initial elution solvent of Et_2O (500 ml) and EtOAc (1000

ml) was used at a flow rate of 1 ml/min to remove non-polar components. The subsequent gradient solvent system [EtOAc–PrOH–H_2O (4:1:1) and (2:1:1)] gave a bioactive alkaloid fraction (1.83 g) positive toward Dragendorff's spray reagent [SiO_2–TLC, $CHCl_3$–MeOH–NH_4OH (2:4:1)]. The alkaloid fraction was subjected to DCCC. DCCC separation was carried out on an apparatus equipped with 300 glass columns [ascending, solvent system $CHCl_3$–MeOH–H_2O–PrOH–NH_4OH (35:65:40:5:1), flow rate 3 ml/h]. The bioactive alkaloid fractions showed three major spots (R_f 0.36, 0.25 and 0.17) on SiO_2–TLC [$CHCl_3$–MeOH–NH_4OH (2:4:1)]. The fractions were chromatographed on a low-pressure SiO_2-column [$CHCl_3$–MeOH–NH_4OH (6:5:1–2:4:1)]. The major spot with an R_f value of 0.36 on SiO_2–TLC [$CHCl_3$–MeOH–NH_4OH (2:4:1)] was purified by HPLC [ODS-SiO_2, MeOH–H_2O (75:25) containing 1.0% NH_4OH] to give arudonine (14 mg).

Arudonine (**1**) HRFAB MS (positive-ion mode): m/z 1000.3454, calc. for $C_{50}H_{81}O_{19}N$ $(M+H)^+$, 1000.3454; CAD (negative FAB); m/z 998 ($[M-H-132]^-$), 886 ($[M-H-132]^-$), 852 ($[M-H-146]^-$), 720 ($[M-H-132-146]^-$), 574 ($[M-H-132-146-146]^-$); UV (methanol) end-absorption; ^{1}H-NMR (ppm in pyridine-d_5); δ 0.72 (d, $J = 5.5$ Hz, 27-CH_3), 0.78 (s, 18-CH_3), 0.95 (s, 19-CH_3), 0.99 (d, $J = 6.7$ Hz, 21-CH_3), 1.41 (d, $J = 6.1$ Hz, rha-2-CH_3), 1.65 (d, $J = 6.1$ Hz, rha1-CH_3), 3.80 (m, 3-H), 4.86 (d, $J = 7.4$ Hz, glc anomeric-H), 5.23 (d, $J = 7.4$ Hz, xyl anomeric-H), 5.23 (m, 6-H), 5.82 (*bs,* rha B anomeric-H), 6.20 (*bs,* rha A anomeric-H). ^{13}C-NMR (ppm in pyridine-d_5); δ 15.6 (C-21), 16.5 (C-18), 18.7 (rha A-6, rha B-6), 19.4 (C-19), 19.8 (C-27), 21.2 (C-11), 30.1 (C-2), 31.1 (C-24), 31.7 (C-15, -25), 32.4 (C-8), 32.6 (C-7), 34.7 (C-23), 37.6 (C-1, -10), 38.7 (C-4), 40.1 (C-12), 40.7 (C-13), 41.6 (C-20), 48.1 (C-26), 50.4 (C-9), 56.7 (C-14), 61.3 (glc-6), 63.6 (C-17), 67.3 (xyl-5), 69.4 (rha B-5), 69.9 (rha A-5), 70.9 (xyl-4), 72.3 (rha B-3), 72.7 (rha A-2), 72.8 (rha B-2), 72.9 (rha A-3), 73.4 (glc-4), 74.0 (rha A-4), 74.2 (rha B-4), 75.2 (xyl-2), 77.7 (glc-5), 77.9 (C-3), 78.8 (C-16, xyl-3), 79.5 (glc-2), 80.7 (glc-3), 98.4 (C-22), 100.5 (glc-1), 101.5 (rha B-1), 102.7 (rha A-1), 104.7 (xyl-1), 121.8 (C-6), 140.8 (C-5).

3.3. Bioassay with lettuce seedlings

Lactuca sativa L., cv Grand Rapids, seeds were purchased from Ferry Morse Seed Company (Mountain View, CA). The assay was performed as previously described (Fukuhara and Kubo, 1991). Lettuce seedlings were placed on a filter paper with 1.3 ml of deionized H_2O in a 5 cm Petri dish. The lengths of lettuce seedling roots were measured after 6 days.

Acknowledgements

The authors are indebted to Dr. G.M. Mungai for plant identification and Mr. A. Chapya for his assistance with plant collection and the initial extraction. KF thanks JT for financial support during his study at UCB.

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