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α- AND β-SOLAMARINE IN KENNEBEC *SOLANUM TUBEROSUM* LEAVES AND AGED TUBER SLICES*

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Abstract—Two major steroid glycoalkaloids, in addition to α-solanine and α-chaconine, were isolated from leaves and aged tuber slices of potato, *Solanum tuberosum* L. var Kennebec. They are glycosides of tomatidenol and have been identified as α- and β-solamarine. The compounds were not found in tuber peel or freshly sliced Kennebec tubers or in 20 other cultivars.

INTRODUCTION

THE PRESENCE of solanine in the potato, *Solanum tuberosum* L., was first reported in 1826.[1] Zwenger and Kind[2] reported that solanine was a glycoside and they named the alkaloidal aglycone solanidine. Subsequently it was found[3,4] that solanine in potato is a mixture of glycosides. Seven glycosides of solanidine have been isolated among which α-solanine and α-chaconine are usually the major components. Glycosides of another steroidal alkaloid, a spirosolenol, were reported by Schreiber,[5] who found a small amount of tomatidenol (**1**) (tomatid-5-en-3β-ol) in an acid hydrolyzate of a glycoalkaloid mixture extracted from potato sprouts. Tomatidenol was also isolated as the major aglycone from a mixture of steroid glycoalkaloids from *Solanum dulcamara* L.[6,7] Fractionation of the mixture led to isolation and characterization of β-solamarine[8] (**2**). The compound showed significant tumor-inhibiting activity against Sarcoma 180 in mice.[9]

In a study[10,11] of the steroid glycoalkaloid content of Kennebec potato in relation to rishitin accumulation and resistance to late blight, two additional major steroid glycoalkaloids (A and B) were detected. These compounds were not observed in leaves of 20 other

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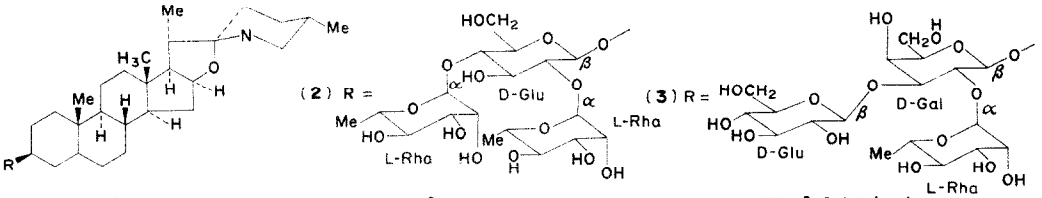
cultivars, and were not detected in the peel of Kennebec tubers even though the total solanine content in the peel is approximately equal to that in the leaf. The compounds accumulated to levels comparable with α-solanine and α-chaconine at the surface of cut Kennebec tuber slices but not slices of the other cultivars. In this work, the two compounds were separated, isolated, hydrolyzed, and their aglycone and sugar compositions determined.



(1) R = HO (2) β-Chacotriosyl (3) β-Solatriosyl

RESULTS AND DISCUSSION

Since the color responses of compound A, which migrates faster than the other three glycoalkaloids on silica gel in all solvents (Table 1), and compound B to Carr-Price[12] and modified Marquis[13] reagents were similar to those of solanidine, α-solanine and α-chaconine, it appeared that A and B were degradative or biosynthetic intermediates between solanidine and its two major glycosides. Sugar analyses of the aqueous fractions of hydrolyzates indicated that A contains 2 mol rhamnose and 1 mol glucose, as does α-chaconine;[14] compound B contains 1 mol each of rhamnose, glucose and galactose, as does α-solanine.[15] The chloroform fraction of hydrolyzates of A and B contained a major com-

TABLE 1. R_f VALUES OF STEROID GLYCOALKALOIDS IN KENNEBEC *Solanum tuberosum* LEAVES

Glycoside*	Solvent system† (A)	(B)	(C)	(D)	(E)	(F)	(G)	(H)	(I)
Compound A ‡ β-solamarine ‡	0·43	0·70	0·63	0·55	0·54	0·55	0·61	0·71	0·44
Compound B	0·32	0·25	0·31	0·17	0·26	0·19	0·50	0·23	0·31
α-Chaconine	0·38	0·63	0·19	0·26	0·36	0·36	0·54	0·54	0·33
α-Solanine	0·23	0·20	0·04	0·01	0·12	0·09	0·41	0·15	0·17

* Visualized after spraying with Carr-Price or anisaldehyde-sulfuric acid reagent. R_f calculations from 5 × 10 cm plates layered with silica gel G (250 μ thick).

† (A) *n*-BuOH–Me_2CO–H_2O (4:5:1), (B) $CHCl_3$–HOAc–MeOH (50:5:45), (C) Me_2CO and MeOH, (D) Me_2CO–MeOH (3:5), (E) EtOAc–HOAc–H_2O (11:2:2), (F) EtOH–HOAc–H_2O (19:1:1), (G) *n*-BuOH–HOAc–H_2O (4:1:1), (H) $CHCl_3$–EtOH–aq. 1% NH_3 (2:2:1, bottom), (I) pyridine–EtOAc–H_2O (1:3:3, upper).

ponent with an R_f different from those of solanidine and solasodine but identical to that of tomatidenol (Table 2). The identity of the aglycone was further confirmed by its elemental analyses, MS analysis, m.p. and m.m.p. with an authentic sample.

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Compound A was confirmed to be β-solamarine by elemental analyses, m.p., R_f values on TLC with various solvent systems and dark-green color formation with anisaldehyde-sulfuric acid reagent. Analytical data for compound B are consistent with that reported for α-solamarine[6,7] (**3**) assuming the sugars of compound B are linked as a α-solamarine.

TABLE 2. R_f VALUES* OF ALKALOID AGLYCONES

Aglycone	Solvent system† (A)	(B)	(C)	(D)	(E)	(F)
Compound C tomatidenol	0·69; 0·77; 0·84‡	0·74	0·80	0·53	0·18	0·23
Solasodine	0·37	0·72	0·75	0·36	0·05	0·06
Solanidine	0·33	0·55	0·70	0·42	0·27	0·09

* Visualized after spraying with Carr-Price reagent, R_f calculations from 5 × 10 cm plates layered with silica gel G (250 μ thick).

† (A) *n*-BuOH–Me_2CO–H_2O (4:5:1), (B) EtOAc–HOAc–H_2O (11:2:2), (C) *n*-BuOH–EtOAc–*iso* PrOH–HOAc–H_2O (7:20:12:7:6), (D) C_6H_6–MeOH (5:1), (E) cyclohexane–EtOAc (1:3), (F) $CHCl_3$–MeOH (19:1).

‡ In a ratio of *ca* 6:1:2. No further separation after 2-D TLC with the same solvent implied no solvolysis involved.

Potato cultivars possessing R genes were introduced by plant breeders in an effort to control late blight,[16] and Kennebec, an R_1 cultivar,[17] is derived from a wild Mexican species, *Solanum demissum* Lindl.[18,19] The steroid glycoalkaloids found in *S. demissum* are demissine and tomatine[20] which contain 5,6-saturated steroidal aglycones and share the same sugar residue, β-lycotetraose. It thus appears from our results that Kennebec inherited only the spirosolane skeleton from *S. demissum* but neither the sugar moiety nor the saturation pattern. Kennebec is the only potato among the R gene cultivars in our investigation which showed such irregularity in its steroid glycoalkaloid spectrum, so that these alkaloids cannot be related to R gene resistance. However, this incident indicates that potentially toxic characters may be introduced when new varieties are brought to the consumer as the result of programs to increase yield, disease or insect resistance or quality of food crops.

EXPERIMENTAL

Extraction of Solanum *alkaloids from Kennebec leaves and aged tuber slices.* Leaf powder (0·5 kg) was extracted (× 2) with 4 l. $CHCl_3$–HOAc–MeOH (10:1:g). The combined extracts were filtered, and evaporated to dryness *in vacuo*. 2 l. each of 2% aq. HOAc and $CHCl_3$ were added to the residue, and the mixture was shaken and allowed to separate. The aq. upper layer was rinsed with 1 l. $CHCl_3$ and reduced to *ca* 300 ml *in vacuo*. The concentrate was centrifuged and the supernatant adjusted to pH 9–10 with NH_3. The mixture was warmed at 80° for 30 min and cooled at 4° for 4 hr. The precipitate was collected by centrifugation (10 min, 15 000 *g*), reprecipitated, washed and dried (yield 4 g). Kennebec tubers were washed with detergent and tap water, surface sterilized by immersing in 70% EtOH for 1 min, surface dried in air, and cut into 1 cm thick slices. The slices were incubated at 18° in glass Petri dishes lined with moistened filter paper for 4 days. The top mm of the aged slices was harvested, lyophilized and extracted as described above.

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TLC procedures. A sample of the crude mixture (leaves or aged tuber slices) was dissolved in MeOH, spotted on silica gel TLC plates and developed in 95% EtOH, and sprayed with satd $SbCl_3$ in $CHCl_3$ (Carr-Price reagent) and heated at 120° for 5 min. Four major purple–red spots appeared, which collectively contributed more than 95% of the total color. The two components with lower R_f were α-solanine and α-chaconine; the two faster moving components were referred to as compounds A and B. For qualitative analyses, precoated silica gel plates with 3% starch as binder were used. About 1–5 μg glycoalkaloids or crude mixture were spotted, and the plates were developed with a range of solvents (Table 1). The developed plates were sprayed with freshly prepared anisaldehyde reagent (0·5 ml anisaldehyde, 9 ml 95% EtOH, 0·5 ml conc. H_2SO_4, and 0·1 ml HOAc) and heated at 120° for 2–5 min till color appeared. A was dark green, B blue, α-chaconine brown, and α-solanine purple–red.

Isolation of A and B. A and B in the crude mixture were separated from α-solanine and α-chaconine on a 48 × 3·5 cm silica gel column (60–200 mesh) eluted with MeOH. A and B appeared in earlier fractions than α-solanine and α-chaconine. Fractions containing A and B were combined and concentrated *in vacuo*. A and B were separated by chromatography on a 38 × 2·7 column of alumina (Woelm neutral, Act. I) using *n*-butanol saturated with H_2O as eluent (300 drops/fraction). Compound A was in fractions 21–55 and compound B in fractions 47–87. Further purification yielded as colorless, amorphous glycosides. *A*. M.p. 268–274° (decomp.), with sintering at 265°. After drying *in vacuo* at 110°/7 for 4 hr (Found: C, 60·24; H, 8·57; N, 1·84. Calc. for β-solamarine monohydrate ($C_{45}H_{73}NO_{15} + H_2O$): C, 61·00; H, 8·53; N, 1·58%). *B*. M.p. 255–260° (decomp.), with sintering at 236°. After drying *in vacuo* at 110°/7 for 4 hr (Found: C, 58·86; H, 8·14; N, 1·69. Calc. for α-solamarine dihydrate ($C_{45}H_{73}NO_{16} + 2H_2O$): C, 58·74; H, 8·44; N, 1·52%).

Identification of hydrolysis products of A and B. A and B were hydrolyzed for 16 hr at 85° in ethanolic MHCl and the products purified and identified by standard procedures. The sugars were analyzed by TLC[21] and GLC,[22] and the aglycone C by TLC (see Table 2). This aglycone was purified by column chromatography on silica gel H-cellulose MN 300 (19:1) and on silica gel H, eluting with $CHCl_3$–MeOH (19:1). It crystallized from acetone as needles, m.p. 238–240°. After drying *in vacuo* at 110° for 4 hr (Found: C, 78·13; H, 10·35. Calc. for tomatidenol ($C_{27}H_{43}NO_2$): C, 78·40; H, 10·48%). Mixture with authentic sample showed no depression in m.p. The MS of compound C and tomatidenol were identical with two intense peaks (*m/e* 138 and 114) and a parent ion of 413.

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