

the method of Kunitz (1947) in the dialyzed guar meal extract. The present study clearly indicates that the low molecular weight fraction contains the highest trypsin inhibitory activity. This is in conformity with the many reports that most trypsin inhibitors isolated from various sources are associated with low molecular weight protein fractions (Liener and Kakade, 1969). Couch et al. (1966) have also reported the presence of a trypsin inhibitor in guar meal and determined its activity by the method of Northrop et al. (1948) using urea-denatured hemoglobin substrate. They found that the level of trypsin inhibition was concentration dependent and the inhibitor was heat labile and apparently not dialyzable indicative of a macromolecular nature.

Hemagglutinin Activity. In the present study it was observed that guar meal extract and the three gel filtration fractions did not show any hemagglutinin activity. However, D'Souza (1972) had reported that one of the protein fractions isolated from the acid extract of guar meal showed a little hemagglutinin activity.

Ammonium Sulfate Fractionation. The ammonium sulfate fractionation of the 1 M NaCl extract of guar gave a S-shaped curve without any sharp breaks. However, three protein fractions which precipitated at 0–20, 20–33, and 33–75% ammonium sulfate concentration, respectively, were tested for homogeneity in an attempt to use this method for separating the protein fractions. Each of these cuts was collected, dissolved in 0.02 M phosphate buffer of pH 7.5, dialyzed against the same buffer, and electrophoresced on polyacrylamide gels. It was observed that none of these fractions gave a single band, suggesting that this procedure could not be adopted for fractionating guar proteins into pure fractions. Further work is in

progress in fractionating guar proteins into pure fractions and studying their physicochemical properties.

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Glycoalkaloid Composition of Wild and Cultivated Tuber-Bearing *Solanum* Species of Potential Value in Potato Breeding Programs

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The glycoalkaloid composition of tubers from a variety of wild and cultivated *Solanum* species has been analyzed qualitatively and quantitatively. These species represent potential breeding stock for the production of new commercial potato varieties, and the glycoalkaloids identified in these species were: tomatine, demissine, α - and β -solanine, α - and β -chaconine, and α -solanine. With the exception of *Solanum acaule*, which runs somewhat high in glycoalkaloid concentration, these clones, based on their glycoalkaloid composition, present no apparent hazard to human health. The conflict in reported data for glycoalkaloid composition of the species *S. acaule* has been resolved.

The introduction of wild *Solanum* species germplasm in potato breeding programs to obtain characteristics such as disease resistance and cold hardiness is being actively studied. The wild Mexican species *S. demissum* has been used for a long time as a source of late blight resistance, and most modern potato cultivars exhibiting such re-

sistance contain germplasm from *S. demissum*. Currently, with the intent of making potatoes a more universal staple, breeders are attempting to develop potato varieties that can be grown in areas of the world where presently available cultivars cannot be grown. Some wild species, because of the environment in which they are found, make good candidates for breeding programs.

The introduction of germplasm from new species must be approached with some caution. Undesirable traits that may not be easily detectable must not be introduced in commercial cultivars. Such undesirable properties should be uncovered in the early stages of a breeding program in

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Table I. Total Glycoalkaloid Content of Selected Species of *Solanum* Tubers

| species | mg/100 g fw ^a |
|-------------------------------------|--------------------------|
| <i>S. tuberosum</i> | 6.4 ^b |
| <i>S. tuberosum</i> cv. SS andigena | 4.9 |
| | 4.0 |
| <i>S. juzepczukii</i> | 15.9 |
| | 11.7 |
| | 19.2 |
| | 41.3 |
| | 18.8 |
| | 46.8 |
| <i>S. ajanhuiri</i> | 7.1 |
| <i>S. acaule</i> | 79.8 |
| | 126 |
| | 53 |
| | 35 |
| | 58.5 |
| <i>S. curtilobum</i> | 29.0 |
| | 21.8 |
| | 18.3 |
| | 3.8 |
| <i>S. stenotomum</i> | 3.6 |
| | 6.1 |

^a fw = fresh weight. ^b Each value represents a means of duplicate analysis of extracts from freeze-dried tuber material.

order to prevent loss of time, money, and effort. The levels and types of glycoalkaloids, a class of toxic compounds common to *Solanum* species and found in present commercial varieties of *S. tuberosum*, present no hazard to human health, but wild species that contain new glycoalkaloids or high levels of glycoalkaloids would be a questionable choice of breeding material in the development of a new cultivar. In the late 1960's, a variety called Lenape, which had *S. chacoense* ancestry (Akeley et al., 1968), was found to exceed what is considered safe glycoalkaloid levels and had to be withdrawn from commerce (Zitnak and Johnston, 1970; Burton, 1974). It is therefore imperative that the glycoalkaloid composition of all species being used in breeding programs be defined. In this report we present the glycoalkaloid composition of *Solanum* species that is presently being examined in breeding programs at the International Potato Center (CIP) in Peru. The glycoalkaloid composition of these species has not previously been determined with the exception of *S. acaule*, for which the literature is confusing as to the actual composition.

MATERIALS AND METHODS

Glycoalkaloid Isolation. Freeze-dried tuber tissue (5 g) of the cultivated species *S. juzepczukii*, *S. ajanhuiri*, *S. curtilobum*, *S. stenotomum*, *S. tuberosum*, and *S. tuberosum* subsp. *Andigena*, and the wild species *S. acaule* was extracted with a mixture of 75 mL of chloroform and 150 mL of methanol in a Waring Blendor for 5 min. The bisolvent mixture was then filtered and the filtrate diluted

with 50 mL of 0.8% aqueous Na₂SO₄. The resultant methanol-water layer was separated, and aliquots of this solution were used for glycoalkaloid analysis.

Quantitative glycoalkaloid content was determined by titration (Fitzpatrick and Osman, 1974). For gas chromatographic analysis the glycoalkaloids were permethylated and separated on a 3% OV-1 column with temperature programming conditions as described by the method of Herb et al. (1975). Both quantitative and qualitative analyses were run in duplicate on aliquots from the same extract. Glycoalkaloid identification was confirmed by thin-layer chromatography (TLC) on silica gel G (Analtech) plates (250 μm) developed with chloroform-methanol (1:1, v/v) saturated with 1% NH₄OH. With this technique, milligram quantities of the individual glycoalkaloids were also isolated and hydrolyzed. The resultant aglycons were identified by comparison of their mass spectra with spectra of authentic compounds. Sugars obtained from the hydrolysis were identified by gas chromatography of the aldonitrile derivatives (Varma et al., 1973).

RESULTS AND DISCUSSION

The total glycoalkaloid concentration (TGA) of each of the species examined is listed in Table I. These values would be considered average (10 mg/100 g fresh weight) to high (>20 mg/100 g fresh weight) for tubers of the size used in these analyses. Most breeders try to maintain levels of glycoalkaloid below 20 mg/100 g tuber fresh weight. The cultivar "Lenape" was found to be as high as 50 mg (Zitnak and Johnston, 1970). High glycoalkaloid levels also have deleterious effects on potato flavor (Sinden and Deahl, 1976). The wide intraspecific variation is not uncommon and probably reflects genetic variability of this material. From these results, it is apparent that some caution should be taken in using the species *S. acaule* in a breeding program. The individual glycoalkaloids and their percentages are shown in Table II. Each one of these compounds has been found in one or more *Solanum* species (Schreiber, 1968); however, commercial varieties of *S. tuberosum* contain only the solanidine glycoalkaloids α-solanine and α-chaconine and their hydrolysis products [recently the tomatidenol glycoalkaloids α- and β-solamarine were reported in incubated slices of the Kennebec variety (Shih and Kuć, 1974)]. Our data represent the first report of glycoalkaloids other than α-solanine and α-chaconine in tuber tissue from cultivated species (Table II). The solamarines have been reported in Kennebec tuber slices but only after incubation and not in freshly ground whole tuber.

The glycoalkaloid composition of the wild species *S. acaule* requires further comment. In addition to *S. acaule* three other "species" (*S. punae* Juz, *S. schreiteri* Buk, and *S. depexum* Juz) have previously been examined for glycoalkaloid composition. These three other "species" are

Table II. Glycoalkaloids of Selected *Solanum* Species

| species | β-chaconine | α-chaconine | glycoalkaloid α-solanine | solamarines ^a | demissine | tomatine |
|----------------------------------|------------------|-------------|-----------------------------|--------------------------|-----------|----------|
| <i>S. ajanhuiri</i> ^b | 3.5 ^c | 39.0 | 57.3 | | | |
| <i>S. curtilobum</i> | | 34.8 | 46.4 | 5.3 | 13.4 | |
| <i>S. stenotomum</i> | 5.5 | 69.8 | 24.7 | | | |
| <i>S. juzepczukii</i> | | 14.0 | 37.8 | 7.7 | 40.4 | |
| <i>S. acaule</i> 1 ^d | | | | | 95.5 | |
| 2 | | | | | 62.1 | 30.9 |
| 3 | | | | | 88.2 | 11.6 |
| 4 | | | | | 64 | 34 |

^a Combined value for α- and β-solamarine. ^b All species are cultivated except for *S. acaule*. ^c Values represent percent of total glycoalkaloids. ^d Four clones of species *S. acaule* were analyzed.

now considered subspecies or synonyms of *S. acaule* (Hawkes and Hjerting, 1969). Whereas one group of workers report tomatine and in some cases solacaoline as the major compounds in this group (Schreiber, 1963), another laboratory (Prokoshev et al., 1952) reports only demissine as the major glycoalkaloid. Our results indicate that tomatine and/or demissine are present in *S. acaule*, but the relative amounts in which they are found will vary between clones; in one clone, tomatine was not present at a detectable level. The possibility exists that either the clones examined by these laboratories were distinctly different in glycoalkaloid composition by chance or that the chromatographic or other techniques used to characterize the compounds could not easily distinguish tomatine from demissine. Also purification procedures necessary for compound characterization may have removed one or the other glycoalkaloid.

Toxicity studies (Nishie et al., 1976) of the glycoalkaloids found in the species listed in Table I indicate that they are of the same order of toxicity as α -solanine and α -chaconine (Nishie et al., 1971, 1975). Therefore, they present no greater hazard than glycoalkaloids now found in commercial potatoes.

Additional glycoalkaloid studies are planned for the analysis of leaves and roots of the species whose tuber tissue was examined in this investigation. Even though these parts of the plant are not consumed as food, the possible role of glycoalkaloids in resistance to insects and diseases and the potential use of potato foliage for animal feed justify the acquisition of this information. Although there can be variation in glycoalkaloid composition in different parts of the plant (Schreiber, 1968), our exper-

ience has shown that the difference is usually quantitative rather than qualitative.

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COMMUNICATIONS

Analysis by Gas-Liquid and Thin-Layer Chromatography of Residual [¹⁴C]Diethylstilbestrol Ear Implants

The unabsorbed parts of 15 [¹⁴C]diethylstilbestrol (DES) ear implants were retrieved at slaughter and were analyzed for total radiocarbon and for DES by gas-liquid chromatography (GLC) and were qualitatively evaluated for DES by three different thin-layer chromatography (TLC) systems. Time in situ and time that [¹⁴C]DES was in the implant form varied among implants. The amount of DES measured by GLC averaged 98.3% of the amount of DES calculated on the basis of total radiocarbon analysis and original specific activity. This percentage did not vary with time and was similar to the percent radiopurity of the [¹⁴C]DES before implantation. These results and the TLC evaluations suggested that the crystalline [¹⁴C]DES, which was 88% of each implant, did not self-decompose during the implant study. The differences between TLC systems demonstrated limitations of TLC for radiopurity evaluation of [¹⁴C]DES.

Radiocarbon-labeled diethylstilbestrol ([¹⁴C]DES) ear implants from the same supplier and formulation were divided between Aschbacher et al. (1975) and Rumsey et al. (1975) for recent studies on the fate of implanted DES in beef steers. These studies related the absorption of [¹⁴C]DES from the implants to the amounts of DES and radiocarbon in tissues, blood, and excreta of the implanted steers. However, interpretation of the radiocarbon data was limited by a small amount of impurities in the ad-

ministered [¹⁴C]DES and the possibility that the highly concentrated [¹⁴C]DES self-decomposed while in the implant form (Rochlin, 1965).

Results from three separate analyses indicated that the [¹⁴C]DES implants used by Aschbacher et al. (1975) and Rumsey et al. (1975) contained a small amount of radiocarbon not associated with DES. The supplier of crystalline [¹⁴C]DES for the implants determined by paper chromatography and by thin-layer chromatography (TLC)