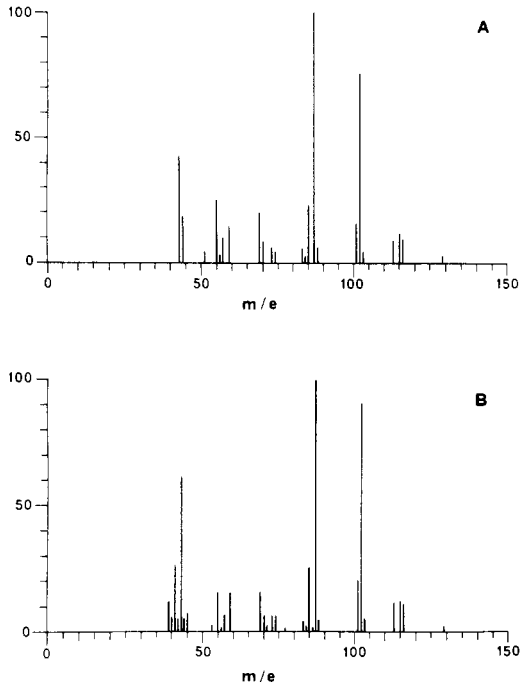

Figure 3. Mass spectra of (A) the methyl ester of the acid isolated from rum, and (B) the methyl ester of 2-ethyl-3-methylbutyric acid.

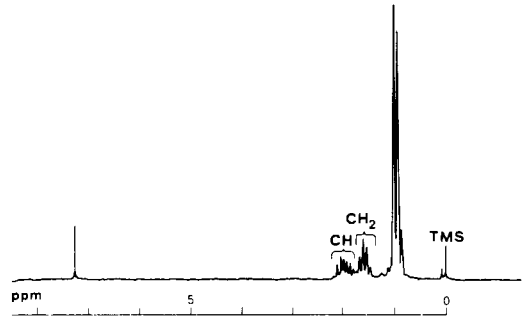

Figure 4. NMR spectrum of 2-ethyl-3-methylbutyric acid.

at wave numbers 1170 and 1150 cm^{-1}. The presence of an ethyl substituent is shown by the rocking of the ethyl CH_2 at wave number 780 cm^{-1} (Nakanishi, 1962).

The identity of the synthesized 2-ethyl-3-methylbutyric acid and the acid isolated from rum was confirmed by the mass spectra of the methyl esters shown in Figure 3. The strong peak of the $(M - 42)^+$ ion at mass number m/e 102 derives from the neutral C_3H_6 fragment being split off from the molecule in a McLafferty rearrangement (Hamming and Foster, 1972). The base peak at mass number m/e 87 is obtained by the subsequent loss of a CH_3 group from the $(M - 42)^+$ ion. The peak at mass number m/e 43 is assigned to the C_3H_7 ion fragment. The relatively weak peak of the $(M - 31)^+$ ion at mass number m/e 113 is a result of the fragmentation of a methoxyl group, and the weak peak of the $(M - 15)^+$ ion at mass number m/e 129 is from the fragmentation of a neutral CH_3 group.

Figure 4 shows the NMR spectrum of the synthesized 2-ethyl-3-methylbutyric acid.

The ultimate source of the acid is not yet known, but its appearance in rums and not in other alcoholic beverages may provide a lead for subsequent investigations.

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Analysis of Mixtures of Solanidine and Demissidine Glycoalkaloids Containing Identical Carbohydrate Units

Mixtures of solanidine-based glycoalkaloids and demissidine-based glycoalkaloids were hydrolyzed under conditions that quantitatively yield solanthrene from the former and demissidine from the latter. The method has been applied to the analysis of potato tuber samples that contain glycoalkaloids differing only in the structure of the aglycone, i.e., solanidine vs. demissidine.

Plants of the *Solanum* genus contain potentially toxic compounds which are carbohydrate derivatives of 3-hydroxysteroidal alkaloids. These compounds are commonly referred to as glycoalkaloids. Their presence in edible plants such as potato, tomato, and eggplant has been of concern because of their toxicity. Although they are usually found at levels that are not toxic, isolated instances of illness and even death (Hansen, 1925) have been ascribed to potato glycoalkaloids.

Much research has been devoted to the identification (Schrieber, 1968) and quantitation (Bretzloff, 1971; Fitzpatrick and Osman, 1974) of glycoalkaloids, particularly in edible plants. Most of the recent qualitative analysis has been done by thin-layer chromatography

(TLC) (Boll, 1962), or by gas–liquid chromatography (GLC) (Herb et al., 1975) for the analysis of permethylated derivatives. The absence in glycoalkaloids of a chromophore absorbing above 210 nm has been a primary obstacle in employing a UV detector in conjunction with high-pressure liquid chromatography, and attempts to apply it to glycoalkaloid analysis have been unsuccessful. Most known glycoalkaloids (Schrieber, 1968) can be separated by either TLC or GLC or a combination of both.

We have recently isolated two glycoalkaloids that could not be separated by chromatographic methods at our disposal, including argentation chromatography. Initially the two compounds were isolated from separate sources. One was characterized as *O*-α-D-glucopyranosyl-(1→2)-*O*-[α-D-glucopyranosyl-(1→3)]-α-D-glucopyranosyl-(1→4)-*O*-galactopyranosylsolanidine which contains a Δ5 double bond in the steroidal aglycone. The other compound was identified as the identical compound *except* that the aglycone is saturated in the Δ5 position. At approximately the same time that these compounds were being characterized, one of the authors (S.S.) found evidence, based on reaction with antimony trichloride on TLC plates, for the presence of mixtures of these two glycoalkaloids in *S. chacoense* tuber samples that were being analyzed as part of a genetic study of glycoalkaloid inheritance. Critical to the study was the determination of the relative amounts of each of these compounds in the samples. We now report a method based on controlled hydrolysis, which gives the desired products followed by GLC analysis, for the determination of these compounds. This method is also applicable to other glycoalkaloids that differ only by a double bond in the Δ5 position.

EXPERIMENTAL SECTION

Glycoalkaloid fractions from potato tuber tissue were isolated by extraction in a Waring blendor with methanol containing a trace of glacial acetic acid (0.01%). Following extraction, the methanolic solution was made basic with concentrated NH_4OH, heated for 1 h at 70 °C, and then refrigerated overnight. The solution was centrifuged and the supernatant was removed. For hydrolysis, a portion of the precipitate (ca. 1–2 mg) was dissolved in 0.5 mL of M H_2SO_4 and heated in a 5-mL screw-capped vial at 95–100 °C for 1.5–2 h. The solution was then cooled, made basic with concentrated NH_4OH, and extracted, in the vial, with 2 × 1.0 mL of benzene. The benzene extracts were combined and concentrated to ca. 0.1 mL. A 5-μL aliquot of this sample was subjected to GLC analysis using the following conditions: column, 3% OV-17 coated on Gas Chrom-Q packed in $^1/_8$ in. × 10 ft glass column; oven temperature programmed from 200 to 280 °C at 6 °C per min; He flow rate, 30 mL/min, FID detector. The identity of the GLC peaks was determined by combined GLC-mass spectrometry using an LKB-9000 mass spectrometer. Glycoalkaloids were separated on silica gel G 250-m plates (Analtech) using $CHCl_3$/MeOH (1/1) saturated with 1% NH_4OH as the developing solvent. Aglycones and their derivatives, e.g., solanthrene, were developed with 5% MeOH in benzene on silica gel G. Mass spectra of purified aglycones were compared with authentic samples.

RESULTS AND DISCUSSION

The hydrolysis of carbohydrate derivatives of the Δ5 unsaturated 3β-ol solanidine (Ia, Figure 1) under the above hydrolysis conditions yields primarily the Δ3,Δ5 diene, solanthrene (Ib), whereas glycosides of demissidine (Ic) upon hydrolysis yield demissidine (Id). Since direct chromatographic methods were unsuccessful in separating Ia from Ic (when R was identical in both cases), we concluded that exploiting this difference in the course of

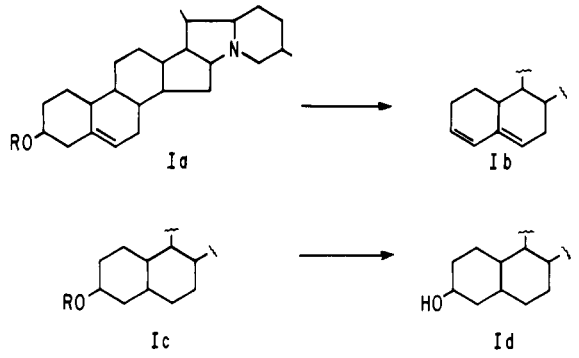

Figure 1. Hydrolysis products of solanidine and demissidine glycoalkaloids (R = sugar moieties).

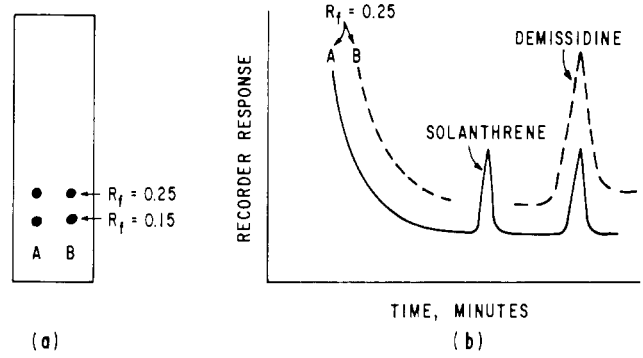

Figure 2. (a) TLC of glycoalkaloids from two *S. chacoense* samples. (b) GLC of aglycones obtained by preparative TLC of R_f 0.25 glycoalkaloids from A and B, followed by hydrolysis.

Table I. Ratio of Solanine (Sol) to Demissine (Dem) Obtained by Hydrolysis of Synthetic Mixtures

Sample	Sol/Dem, calcd	Sol/Dem, obsd	% recov.
1	10/90	13/87	89
2	25/75	27/73	91
3	50/50	50/50	87
4	90/10	85/15	89

hydrolysis offered the best method for the quantitative analysis of two such similar compounds in the presence of eath other. The primary problem was to define hydrolysis conditions that would give the desired products in high yield. Hydrolysis conditions that were too mild would lead to incomplete hydrolysis of the glycoside and, in the case of solanidine glycoalkaloids, incomplete conversion to solanthrene. Hydrolysis conditions that were too vigorous could result in the destruction of solanthrene and in dehydration products of demissidine. After examining a variety of hydrolysis conditions including time, temperature, acid strength, and acid type, we concluded that the optimum hydrolysis condition for obtaining the desired products is: 2 N H_2SO_4 at 95–100 °C for 1.5–2 h. The accuracy of the method was demonstrated through the analysis of a series of known mixtures of demissine and solanine. The former contains the demissidine aglycone, the latter the solanidine aglycone. It would have been desirable to use glycoalkaloids containing identical carbohydrate moieties; however, no such combination was available in sufficient quantity. Acceptable ratios of solanidine type to demissidine type were obtained, and the overall yield (based on GLC peak areas) indicated the method to be almost quantitative (Table I).

The method has been applied to the analysis of glycoalkaloid fractions from tuber samples mentioned above. Figure 2a shows a typical TLC separation of two of the

samples in the study. Under any chromatographic conditions we investigated, similar results were obtained, i.e., the glycoalkaloids in both samples had identical R_f values, and only by the use of $SbCl_3$ as spray reagent was it possible to detect that different glycoalkaloids were present in the samples, by the intensity of violet color development. GLC traces of the hydrolyzates of R_f 0.25 from both samples are shown in Figure 2b. One sample was composed of demissidine glycoalkaloids almost exclusively. The other sample was a mixture of demissidine and solanidine glycoalkaloids.

Anomalous peaks that were found in the GLC analysis in most cases appeared to occur because of lack of complete removal of lipids in the initial isolation of the glycoalkaloid fraction; these peaks did not interfere with the analysis but could be removed by washing the glycoalkaloid precipitate with chloroform.

Although we investigated only glycoalkaloids containing the aglycones solanidine and demissidine, the method should also be applicable to such pairs as tomatidine (Δ5 saturated) and tomatid-5-en-3-ol (Δ5 unsaturated).

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Polyamines in Green and Roasted Coffee

Three polyamines, putrescine (1,4-diaminobutane), spermine (α,δ-bis(γ-aminopropylamino)butane), and spermidine (α-(γ-aminopropylamino)-δ-aminobutane), have been isolated from green coffee beans and identified by thin-layer chromatography. During the roasting process these polyamines are degraded, probably being precursors of coffee flavor formation. However, coffee beans of the same variety and harvested in the same year, which produced different beverage quality, showed similar polyamine content. No nitrite could be detected in the green bean, which would avoid nitrosamine formation during the roasting process.

Polyamines are widely distributed in plants (Smith, 1971), and they have also been found in many seeds (Moruzzi and Caldarera, 1964). Recently, Wang and his co-workers (Wang et al., 1975) showed that putrescine, spermine, and spermidine have a threshold level (50% correct response) between 10^{-4} and 10^{-5} M in water. The odor of these polyamines in water is a putrid one, but if polyamines form salts with glutamic acid, the flavor of soy sauce could be improved (Udo, 1931, 1932).

The price of coffee beans depends on the flavor of the infusion of the roasted beans, and it would be of interest to know if polyamines exist in green coffee, their behavior during the roasting process, and if coffees with different qualities of beverage within the same variety have the same polyamine content.

MATERIALS AND METHODS

Commercial coffee beans (*Coffea arabica* L. var. Mundo Novo) were harvested in 1974 from different regions of the State of São Paulo and Minas Gerias, Brazil. After roasting and brewing, they were classified by professional tasters with regard to the quality of the beverage as either Soft (mild flavor) or Rio (strong medicinal flavor). Samples not well characterized were discarded.

The beans (500 g) were roasted in a commercial roaster at 240 °C for 12 min. One sample was taken at 9–10 min, which represents the light roast (9–10% weight loss), and the other at 12 min, which represents the dark roast (15–17% weight loss).

Polyamine Determination. Coffee beans were ground in a Microbroyeur Quantitatif Dangoumau (Prolabo, Paris, No. 7499.02), and 5 g of the powder (10 g for the roasted) was immediately extracted with 60 mL of 5% trichloroacetic acid by shaking for 2 h. After centrifugation, the total volume and the supernatant were measured, and the residue was discarded. Successive extractions with 5% Cl_3CCOOH proved unnecessary if the volume retained by the residue was taken into account for correction.

Extraction with 75% ethanol acidified with 4 N HCl to pH 4.0 removed the same amount of putrescine as 5% Cl_3CCOOH, but the recovery of spermine and spermidine was much smaller. Ethanol without acidification extracted less putrescine than 5% Cl_3CCOOH.

After preliminary purification of the supernatant with Dowex-50 W-X8 (H^+) resin (20–50 mesh) according to Smith (1970), the amines were dansylated and chromatographed using 250-μm thick Kieselgel G (Merck) commercial TLC plates and cyclohexane/ethyl acetate (3:2 and 1:1) as solvent.

Putrescine, spermine, and spermidine were estimated by fluorometry of their dansyl derivatives in TLC plates using a Vitration TLD 100 densitometer (Smith, 1973). Two replicates were made for each coffee sample.

The identity of the polyamines was checked by cochromatography and by comparing with pure compounds in the following solvent systems: cyclohexane/ethyl acetate (3:2), cyclohexane/diethyl ether (1:9), and cyclohexane/chloroform (1:19) by using the same TLC plates mentioned herein.

The following R_a (relative to ammonia) values were recorded for the dansyl derivatives: putrescine, 0.81; spermidine, 0.60; and spermine, 0.44 (solvent system,