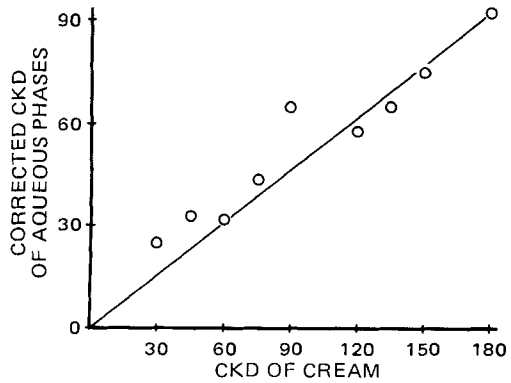

Figure 2—*Relationship between the critical kill dilutions of cream systems and the corrected critical kill dilutions of the partitioned aqueous phases.*

factory emulsion stability. A satisfactory combination of these two properties was not possible in the cream systems containing no hydrocarbon or 15% mineral oil.

Effect of Percent Hydrocarbon—The effect of the concentration of hydrocarbon on the critical kill dilution of creams emulsified with the myristyl quaternary ammonium compound and containing either cetyl or stearyl alcohol was evaluated (Table VII). The critical kill dilutions of the creams were similar in rank order to those observed in the critical kill dilution evaluation of the partitioned systems. The critical kill dilutions increased with increasing hydrocarbon concentration, with the stearyl alcohol creams being more effective than the corresponding cetyl alcohol base creams.

The white petrolatum creams showed a larger increase in critical kill dilution compared to the creams containing mineral oil of the same concentration. The increase may again be attributed to the greater concentration of quaternary ammonium compound present in the aqueous phase of the white petrolatum creams.

The relationship between the critical kill dilution of a cream and its corresponding aqueous phase was observed throughout the previous study. Figure 2 compares the relationship by pairing the corrected critical kill dilutions for the partitioned aqueous phases with the corresponding cream formula. The critical kill dilutions of the creams were ranked, and the corresponding corrected critical kill dilutions of the corresponding aqueous phases were averaged. From the data obtained, it appeared that on the average a cream was about twice as effective as the corresponding partitioned aqueous phase. The correlation technique appears to offer a method of correlating the bactericidal effectiveness of a cream and *in vitro* conditions, such as in the partitioning studies. The result also indicates that the nonaqueous phase also possesses antibacterial activity; otherwise, the slope of the plot would equal unity.

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* Present address: Pharmaceutical Manufacturing Development, The Upjohn Co., Kalamazoo, MI 49001.

[x] To whom inquiries should be directed.

Determination of Solasodine in Fruits of *Solanum* Species

LEHEL TELEK

Abstract □ A potentiometric nonaqueous titration procedure was developed for the quantitative determination of solasodine in fruits of the *Solanum* species. The steroid glycoalkaloids were extracted from freshly harvested fruits with 2% acetic acid and methanol. After hydrolysis, the common aglycone solasodine was extracted with benzene. An aliquot was mixed with an equal volume of acetone and titrated potentiometrically with 0.005 *N* perchloric acid in dioxane, using glass and silver electrodes for the determination.

Keyphrases □ Solasodine—potentiometric analysis in fruits of *Solanum* species □ *Solanum* species—potentiometric analysis of solasodine in fruits □ Potentiometry—analysis, solasodine in fruits of *Solanum* species □ Steroids—solasodine, potentiometric analysis in fruits of *Solanum* species

The consumption of pharmacologically active steroids is increasing significantly (1). The use of steroidal contraceptives will spread with the pressing need for birth control. In the growing demand for raw materials, solasodine is the most competitive in supplementing diosgenin in the partial synthesis of steroid hormones (2). The special

advantages of solasodine are that all manufacturing processes based on diosgenin apply to it without any changes (3) and that its plant sources, some *Solanum* species, are easy to cultivate in the temperate zone.

Schreiber (4) reviewed the *Solanum* species that contain steroid glycoalkaloids in various plant parts. *S. laciniatum* Ait. is utilized industrially, and it is extensively cultivated in the Soviet Union (5) and New Zealand (6). In numerous *Solanum* species, the active ingredient is concentrated in their fruits. In Puerto Rico, *S. mammosum* L. and *S. khasianum* C. B. Clarke were studied as potential plant sources of steroid hormones[1].

In agronomic and plant biochemical research, a fast, reliable method was required for quantitative determination of solasodine in fruits of the *Solanum* species. Published procedures for the determination of solasodine in dried leaves of *S. laciniatum* include preparative gravimetry (7), colorimetry (8–11), titration in nonaqueous media (12, 13), and potentiometric titration in aqueous media (14). However, none of these methods is suitable for analyzing *Solanum* fruits.

The present report describes the direct quantitative determination of solasodine in fresh fruits, without drying, from the *Solanum* species grown in Puerto Rico in 1970 and 1971.

EXPERIMENTAL

Apparatus—An automatic system was used for the titrations[2]. A glass electrode[3] and a laboratory-made silver electrode, consisting of a folded silver strip 6.4 mm wide, 10 cm long, and 0.16 mm thick[4], were used. The sample was mixed using a magnetic stirrer and a 12.5-mm polytef[5]-coated stirring bar.

Reagents—Reagent grade acetic acid, benzene, methanol, and acetone were used without purification. Reagent grade dioxane was purified by the method of Chatten *et al.* (15) to eliminate browning with perchloric acid.

The 0.005 *N* perchloric acid titrant was prepared by dissolving 5 ml of 72% perchloric acid (reagent grade) in 100 ml of dioxane. From this 0.5 *N* $HClO_4$ stock solution, 5 ml was made up to 500 ml with purified dioxane. The perchloric acid titrant was standardized with atropine base[6] as a reference standard, which was purified by recrystallization from acetone and dried at 103°; its purity was checked by TLC (16).

Procedures—The fruits of *S. mammosum* and *S. khasianum* were harvested as soon as they reached a transition green-to-yellow color and were immediately extracted.

Extraction—Each of four or five fruits of *S. mammosum* or 20–25 fruits of *S. khasianum* of the same degree of maturity, collected from a single plant, was cut into four pieces with a sharp knife in a petri dish. The pieces were homogenized in a blender until they formed a thick, uniform slurry. The knife and dish were wiped with a small filter paper, which was added to the blender. From the homogenate, about a 10-g sample was weighed into a tared dish for determining the moisture content by drying to constant weight at 103°.

The glycoalkaloids were extracted by weighing 50 g of the homogenate in a beaker, transferring it quantitatively into a homogenizer[7] with 100 ml of 2% aqueous acetic acid, and mixing for 5 min. The slurry was centrifuged for 10 min, the supernate was decanted into an erlenmeyer flask, and the centrifuged solids were reblended and reextracted with 100 ml of methanol. After centrifugation, the methanol extract was combined with the acetic acid extract and again centrifuged. The clear solution was transferred into a 250-ml volumetric flask and diluted to volume with methanol.

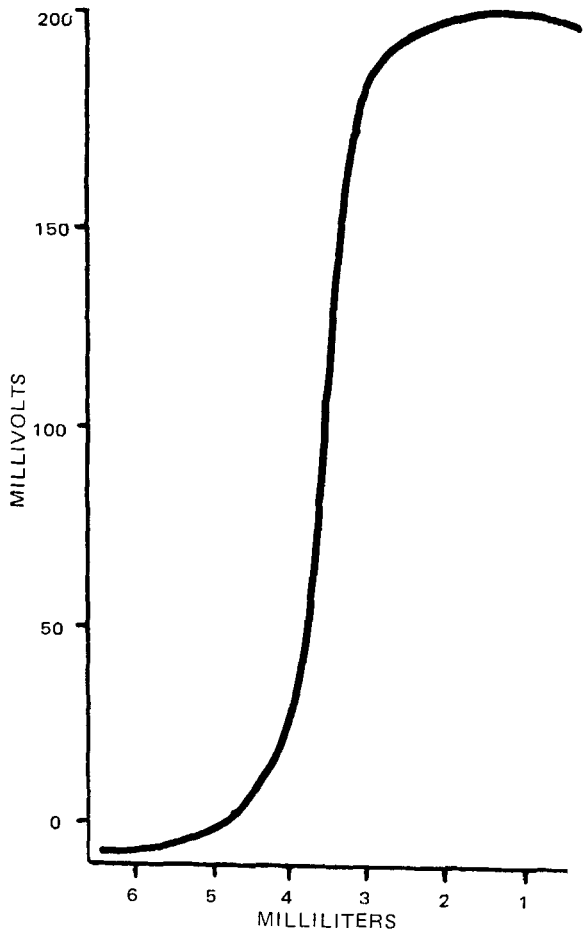

Figure 1—*Titration curve of solasodine.*

Hydrolysis—Duplicate 50-ml aliquots of the extracts were measured into 125-ml erlenmeyer flasks equipped with glass-tube condensers. Concentrated hydrochloric acid (5 ml) was added, and the mixture was heated in a boiling water bath for 2 hr. After cooling, 20 ml of 25% aqueous sodium hydroxide was added, and the mixture was heated in a water bath for 1 hr. After cooling, the content of the flask was transferred quantitatively into a separator and extracted with 25, 15, and 10 ml of benzene, consecutively. The pooled benzene extracts were dried with anhydrous sodium sulfate, transferred into a 50-ml volumetric flask, and brought to volume with benzene.

Twenty-five milliliters of the benzene extract was pipetted into the titration vessel, and 25 ml of reagent grade acetone was added. The extract was stirred at medium speed with a polytef stirring bar. The titration was monitored with a recorder by setting the electrode potential amplifier to the 100-mv scale and the recorder speed to 12.7 cm/min. Initially, the titrant was added rapidly; but as the first derivative curve began to appear, the rate of addition of titrant was decreased.

The percent of solasodine was calculated from:

$$\text{solasodine (\%)} = \frac{VF2.069 \times 100}{W_d} \qquad \text{(Eq. 1)}$$

where V = milliliters of titrant, F = normality factor of titrant, 2.069 = milliequivalents of solasodine, and W_d = weight of sample calculated as dry weight:

$$W_d = W_f - (W_f \times \%M) \qquad \text{(Eq. 2)}$$

where W_f = weight of fresh sample and $\%M$ = moisture content of fresh sample.

RESULTS AND DISCUSSION

Previously published analytical methods were developed for the evaluation of the solasodine content of dried and milled leaves of *S. la-*

[1] The plant materials used in this investigation were obtained by the Mayaguez Institute of Tropical Agriculture and identified by taxonomists of the Agricultural Research Service, U.S. Department of Agriculture, Plant Research Center, Beltsville, Md. Voucher specimens have been sent to the Herbarium, U.S. National Arboretum, Washington, D.C.

[2] Mettler DV 101 buret, DK 10 electrode potential amplifier, DV 103 command module, and Kentron 1110 pulse drive recorder.

[3] Beckman 1190-80.

[4] A. Thomas catalog No. C695-Q55.

[5] Teflon (du Pont).

[6] ICN Life Science Group, K and K Laboratories, Plainview, NY 11803.

[7] Omni mixer, Sorvall, Inc., Newtown, Conn.

Table I—Analysis of Isolated Solasodine

Sample[a]	Origin	End-Point Indication	Solasodine, %	Number of Analyses
Crude	*S. mammosum*	Potentiometric	86.5 ± 0.8	10
Crude	*S. khasianum*	Potentiometric	91.2 ± 0.6	10
Recrystallized	*S. mammosum*	Potentiometric	99.8 ± 0.4	10
Recrystallized	*S. khasianum*	Potentiometric	99.8 ± 0.4	10
Purified (preparative TLC)	*S. mammosum*	Potentiometric	100 ± 0.3	3
Purified (preparative TLC)	*S. mammosum*	Color indicator[b]	100 ± 0.4	2

[a] Every sample was heated to 105° for 2 hr before analysis. [b] From Ref. 13.

ciniatum. To achieve homogeneity of the sample, 250 g of dried leaves had to be milled through a fine mesh screen. The efficiency of extraction was inversely proportional to the particle size (17). To obtain a homogeneous sample using previous methods, serious problems would have been encountered. The steroid glycoalkaloids are not uniformly distributed in the fruit. The sap around the seed is very rich in the active principle, whereas the skin and seeds contain little or none.

It is difficult to dry a whole fruit quickly, and slow drying may cause decomposition. Moreover, the dried fruit cannot be minced because the high glycoalkaloid-containing sap tends to burn on the blades of the mill. Homogenization of fresh fruits yielded the best results. Extraction of freshly harvested fruits with weak acids and the subsequent precipitation of pectinous substances by methanol resulted in transparent yellow-colored solutions, free of suspended solids. The *Solanum* glycoalkaloids were removed quantitatively from the fruits.

In previously reported methods, the glycoalkaloids were isolated prior to hydrolysis. This step was unnecessary in the present procedure. After acid hydrolysis of the fruit extract, the solasodine was extracted with benzene from the hydrolysate.

Other methods suggested colorimetric techniques for determining solasodine based on its reaction with amphi-indicators such as methyl orange and tropaeoline 000 (8). However, these procedures are feasible only with purified compounds or colorless sample solutions.

The widely used gravimetric method, based on the weighing of a dried precipitate of solasodine hydrochloride, is not specific for solasodine and is subject to interference by inorganic coprecipitates.

The nonaqueous titration of secondary amines of alkaloids with 0.001 *N* *p*-toluenesulfonic acid, using dimethyl yellow as an indicator, is well established (18). It was applied (12, 13) to determine solasodine in leaves of *S. laciniatum*. When this method was applied to the colored fruit extracts, it was practically impossible to detect the transition color of the dimethyl yellow indicator. The chloroform solution of solasodine was also unstable, and the normality of *p*-toluenesulfonic acid in chloroform changed on standing.

Potentiometric determination of the end-point of the chloroform solutions of extracts of *Solanum* fruit titrated with *p*-toluenesulfonic acid also gave erratic results. Fritz and Burgett (19) titrated amines in acetone, using perchloric acid in dioxane as the titrant. When solasodine was titrated by this method, using calomel and glass electrodes, the resulting curves changed after each titration. By replacing the calomel electrode with a small strip of silver foil, titration of benzene extracts mixed with equal volumes of acetone gave good results. The titration curve and its first derivative curve are shown in Figs. 1 and 2.

The curves of purified solasodine and plant extracts were reproducible after repeated titrations. As a check of the procedure, the peak of the first derivative curve correlated well with the transition color of the added dimethyl yellow indicator when titrating colorless solutions of solasodine samples.

Fritz (20) recommended potassium acid phthalate in glacial acetic acid solution as a reference for standardization of perchloric acid in dioxane solution, using methyl violet as the indicator. The observation of the transition color of this indicator is very subjective, and potentiometric end-point determination of this system with glass and silver electrodes was unsuccessful.

Therefore, atropine was chosen for a reference standard. The purity of atropine was checked by TLC and by titration using the method of Gyenes (18). The titration of atropine base in benzene and acetone solution with perchloric acid in dioxane gave a curve similar to that of solasodine, and the first derivative curve showed a sharp end-point. The transition color of the dimethyl yellow indicator appeared exactly when the recorder reached the maximum of the first derivative curve.

The variable speed command module of the titration apparatus permitted the rapid addition of the bulk of the titrant; as the end-point was approached, the addition could be suitably slowed down. In this way, error due to temperature fluctuations could be minimized (21).

Titrations of crude, recrystallized, and chromatographically purified solasodine samples gave precise results. Titration of crystalline solasodine by the method of Bite (13) yielded the same result as that with the potentiometric determination (Table I).

The merits of this described method are illustrated in Table II. For the method development experiments, a composite sample was prepared of randomly picked yellow-green fruits of *S. mammosum* of different shapes. With the described extraction procedure of fresh samples, the average deviation in six assays was only 1.89% when the end-point was determined potentiometrically. However, when the methyl yellow indicator was used for the end-point determination, the average deviation of the results of four measurements was 18.4%. This deviation was further increased when the analytical samples were prepared by soxhlet extraction of dried and milled fruit (22). The titration of dark-colored samples was difficult using the methyl yellow indicator, and the average

Figure 2—*First derivative curve of solasodine.*

Table II—Analysis of a Composite Sample of Yellow-Green Fruits of *S. mammosum* for Method Development

Moisture Content[a], %	Extraction Method[b]	End-Point Indication	Solasodine Content, %	Number of Determinations
80.8	I	Potentiometric	1.06 ± 0.02	6
80.8	I	Color indicator[c]	0.98 ± 0.18	4
Dried	II	Potentiometric	0.94 ± 0.06	4
Dried	II	Color indicator[c]	0.92 ± 0.32	4
Previously extracted[d]	I	Potentiometric	2.04 ± 0.04	4

[a] Determined by drying to constant weight at 105°. [b] Extraction Method I is as outlined in this paper; Method II uses dried fruit and methanol extraction (22). [c] From Ref. 13. [d] Crystalline solasodine, 0.2 g, was added to 10 g of extracted sample.

Table III—Analyses of Fruits of *S. khasianum* and *S. mammosum*[a]

Fruit	Color	Shape	Moisture Content[b], %	Solasodine Content, %
S. khasianum	Green	Round	76.8	0.82 ± 0.04
S. khasianum	Yellow	Round	80.4	1.12 ± 0.06
S. mammosum	Yellow-green	Pear	79.1	1.05 ± 0.04
S. mammosum	Yellow-green	Round	81.9	0.99 ± 0.02
S. mammosum	Yellow-green	Pear with nipples	80.2	0.91 ± 0.03

[a] In all cases, the extraction method used was the one described in this report, and the end-point was determined potentiometrically (n = 3). [b] Determined by drying to constant weight at 105°

error of four titrations was 34.8%. Titrating the same sample with potentiometric end-point detection gave an average deviation of 6.4% in four measurements.

The recovery of added crystalline solasodine to an exhaustively extracted *S. mammosum* sample was quantitative within the experimental error of 2%, demonstrating the reliability of the extraction procedure outlined in this report.

Table III summarizes the results of solasodine determinations performed according to this procedure. Freshly harvested fruits of *S. khasianum* at different ripening stages and fruits of *S. mammosum* of different shapes were analyzed. Experimental errors were at the level previously experienced in the method development assays.

These analyses of *S. mammosum* and *S. khasianum* samples yielded more realistic values than those reported in the literature. Perez-Medina *et al.* (22) found 3.5–4% glycoalkaloids in *S. mammosum*. Maiti and Mathew (23) found 5% solasodine in *S. khasianum*. These investigators used the gravimetric method, and their results showed large variations and unusually high values when compared to solasodine levels found in other *Solanum* species. The present analyses showed alkaloid contents of 0.82–1.12% for these plants (Table III).

The easy operation of the titrating apparatus and the recorded end-point determination method should permit technicians to analyze single samples with sufficient accuracy for field evaluations.

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Antimicrobial Action of Compound 48/80 against Protozoa, Bacteria, and Fungi

J. F. LENNEY *x, W. A. SIDDIQUI ‡, J. V. SCHNELL ‡, E. FURUSAWA *, and G. W. READ *

Abstract □ Compound 48/80 inhibited the growth of protozoa, bacteria, and fungi but had no effect on the multiplication of viruses. All susceptible organisms were inhibited by 10 μg/ml of crude compound 48/80, and some were inhibited by as little as 0.1 μg/ml. Against *Tetrahymena pyriformis*, this drug was seven times more potent than quinine. Separation of compound 48/80 into different fractions indicated that some antimicrobial activity could be separated from the histamine-liberating activity. It was found that compound 48/80 is not surface active at 500 μg/ml.

Keyphrases □ Compound 48/80—antimicrobial activity evaluated in protozoa, bacteria, fungi, and viruses □ Antimicrobial activity—compound 48/80 evaluated in protozoa, bacteria, fungi, and viruses □ Phenethylamine polymers—compound 48/80, antimicrobial activity evaluated in protozoa, bacteria, fungi, and viruses □ Polymers of phenethylamine—compound 48/80, antimicrobial activity evaluated in protozoa, bacteria, fungi, and viruses

In 1949, a family of polymers that lowered blood pressure was synthesized from *p*-methoxyphenethylmethylamine and formaldehyde (1). It was proposed that the products were various polymers of phenethylamine and that the most potent oligomer in the mixture was possibly the trimer. A widely distributed batch of this product was