Solasodine Stability under Conditions of Saponin Hydrolysis

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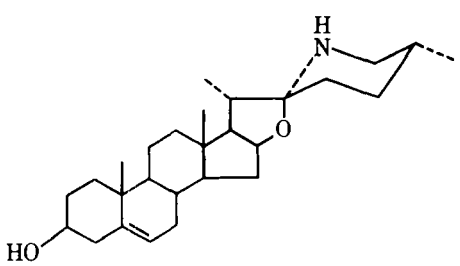
Received August 29, 1977, from the *Department of Pharmacognosy, School of Pharmacy, The Hebrew University of Jerusalem, Jerusalem, Israel.* Accepted for publication November 8, 1977.

Abstract □ The extent of formation of solasodiene from solasodine upon treatment with hydrochloric acid under various conditions was determined. The diene formed was assayed by using the characteristic extinction at 236 nm. Diene formation was effected by the acid concentration and the boiling point of the solvent used. Optimal conditions for the hydrolysis of solasodine glycosides are suggested.

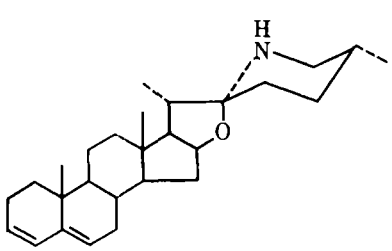
Keyphrases □ Solasodine—formation of solasodiene on hydrolysis with hydrochloric acid under various conditions □ Solasodiene—formation from solasodine on hydrolysis with hydrochloric acid under various conditions □ Hydrolysis—solasodine, formation of solasodiene on treatment with hydrochloric acid under various conditions □ Stability—solasodine, formation of solasodiene on hydrolysis with hydrochloric acid under various conditions □ Saponins—solasodine glycosides, formation of solasodiene on hydrolysis with hydrochloric acid under various conditions

Recently (1), solasodine (I) has become a highly valued compound, being a competitor to diosgenin as a precursor of pharmacologically active steroids. Solasodine occurs in different *Solanum* species in glycosidal combination, *i.e.*, as the aglycone of various saponins, and is obtained from the plant extract by acid hydrolysis. Because of the growing demand for raw materials for the synthesis of steroidal hormones, it is important to choose conditions for obtaining optimal yields of solasodine from the plant material.

While deriving solasodine from *S. incanum* (2), it was apparent that the yield was highly influenced by the experimental conditions. Examination of the crude solasodine, obtained after acid hydrolysis of the plant extract, showed that the sapogenin was accompanied by various quantities of a less polar compound (3). This compound proved to be the artifactual dehydration product solasodiene (II). Acids can decompose 3-hydroxy-Δ^5-steroids to the dehydrated $\Delta^{3,5}$-dienes (4).



I



II

The stability of diosgenin under various hydrolytic conditions was investigated thoroughly (4–6). However, no analogous research has been carried out with respect to solasodine, although solasodine is known to give the corresponding diene (7–9).

This paper reports the effect of the acid concentration as well as of the solvent system on solasodine hydrolysis.

RESULTS AND DISCUSSION

Solasodiene (II) was isolated from the reaction of solasodine (I) with ethanolic hydrochloric acid. The compound was identified by its melting point, mass spectrum (M^+ 395), and UV and IR spectra. This compound was identical with the less polar compound obtained in all of the acid-catalyzed reactions of solasodine (same R_f values on TLC, developed in three different solvent systems).

Since the solasodiene obtained after solasodine hydrolysis possesses the characteristic diene chromophore, which is absent in the parent compound, it was possible to monitor its formation by UV spectroscopy. This absorbance followed Beer's law and was scarcely influenced by the coexistence of the corresponding Δ^5-3-ol, *i.e.*, solasodine. The yield, *i.e.*, the extent of the dehydration of solasodine, was evaluated from the absorbance at 236 nm, the molar absorption being 2.07×10^4.

The yields of solasodiene obtained on refluxing solasodine under various conditions are summarized in Table I. The yield of the diene was highly influenced by the acid concentration and by the type of alcohol used. The higher the boiling point of the alcohol, the more diene was obtained. High boiling alcohols, *i.e.*, 1-propanol, bp 97°, and 1-butanol, bp 116°, were used since solasodine hydrochloride is extremely insoluble in water and aqueous alcoholic solutions, the solvents usually used for the extraction and hydrolysis of plant saponins. Even when a low boiling alcohol was used with a high acid concentration (2 *N*), the diene was obtained in a considerable yield.

It seems that the most suitable condition for the saponin hydrolysis is the use of nonaqueous low boiling alcohols and acid concentrations not exceeding 1 *N*.

EXPERIMENTAL

Starting Compound—A commercial sample of solasodine was purified by silica gel column chromatography, using chloroform–methanol

Table I—Yield of Solasodiene from Solasodine [a] upon Boiling [b] with Acid under Various Conditions

Alcohol	Hydrochloric Acid Concentration, *N*	Absorbance at 236 nm [c]	Yield of Solasodiene from Solasodine, %
Methanol	0.5	0.075	1.4
	1	0.13	2.4
	2	0.66	12
Ethanol	0.5	0.09	1.6
	1	0.20	3.7
	2	1.34	25
1-Propanol	0.5	0.29	5.4
	1	0.65	12
	2	2.02	37.5
1-Butanol	0.5	0.34	6.3
	1	1.04	19
	2	2.85	54

[a] Pure solasodine (5 mg) dissolved in 10 ml of alcohol to which concentrated hydrochloric acid was added to give the required concentration. [b] Reflux for 3 hr. [c] UV spectra were measured on Varian Techtron model 635.

0022-3549/78/0800-1169$01.00/0

(40:1) as the eluting solvent. The compound was further purified by crystallization from acetone.

TLC—Silica gel 60 F_{254}-precoated aluminum sheets were used with chloroform–methanol (10:1), giving R_f 0.62 for I and R_f 0.8 for II. The spots were visualized by spraying with the following mixture: anisaldehyde (21 ml), phosphomolybdic acid, 10% in methanol (5 ml), acetic acid (45 ml), sulfuric acid (22.5 ml), and methanol (430 ml). The plates were heated to 120–125° and development of color was observed.

Isolation of Solasodiene—Solasodine (400 mg) was dissolved in 100 ml of ethanol, and 24 ml of concentrated hydrochloric acid was then added. The resulting solution was refluxed for 3 hr, cooled, and neutralized with aqueous ammonia, and the methanol was evaporated. The residue was extracted with ether, and the combined extracts were washed with saturated aqueous sodium chloride and then dried over magnesium sulfate. After evaporation of the ether, the mixture was separated by silica gel column chromatography, using chloroform–methanol (40:0.5) as the eluting solvent. Crude solasodiene (25%) was obtained. Crystallization from acetone gave pure solasodiene, mp 173°.

General Procedure for Hydrolysis—Solasodine (0.005 g) was dissolved in methanol, ethanol, 1-propanol, or 1-butanol, and then concentrated hydrochloric acid was added to give the required acid concentration (0.5, 1, and 2 *N* for each alcohol used). The total volume of each solution was 10 ml. The solution was refluxed for 3 hr and neutralized with aqueous ammonia. After evaporation to dryness in a vacuum, a second portion of aqueous ammonia was added, making the reaction mixture alkaline. This mixture was then extracted with ether, and the combined extracts were washed with saturated sodium chloride, dried over magnesium sulfate, and evaporated to dryness. The residue was then diluted with methanol to the required volume for UV spectroscopic reading.

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Analysis of Cefazolin in Serum or Urine

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Received March 7, 1977, from *Pfizer Central Research, Pfizer Ltd., Sandwich, Kent CT13 9NJ, England.* Accepted for publication November 1, 1977.

Abstract □ A method was developed to determine cefazolin in serum or urine. The drug is extracted from serum or urine with ethyl acetate, separated by TLC, and determined by fluorescence quenching densitometry. The method was developed to study the pharmacokinetics of the compound in humans.

Keyphrases □ Cefazolin—TLC–densitometric analysis in biological fluids □ TLC–densitometry—analysis, cefazolin in biological fluids □ Antibacterials—cefazolin, TLC–densitometric analysis in biological fluids

To carry out pharmacokinetic studies on cefazolin, 7-[1-(1*H*)-tetrazolylacetamido]-3-[2-(5-methyl-1,3,4-thiadiazolyl)thiomethyl]ceph-3-em-4-carboxylic acid (1), an assay was developed to determine antibiotic concentrations in serum and urine. The method provides a convenient alternative to other published procedures (1, 2).

EXPERIMENTAL

Reagents—Ether[1], ethyl acetate[1], chloroform[1], acetic acid[2], 1 *M* HCl, and 0.2 *M* HCl were used.

TLC–Densitometry—Precoated TLC plates[3] (20 × 20 cm) were developed in glass tanks. Solutions were applied to the thin-layer plates using an automatic plate spotter[4]. Plates were scanned using a densitometer[5]. The settings on the instrument were: mode, log −; span, 980; level, e5; damping, 2; rotating-spot diameter, 8 mm; scan speed setting, 3; and chart speed setting, 5.

Analysis of Samples—A sample of serum or urine (1.0 ml diluted 0–500-fold) was pipetted into a 10-ml stoppered tube. The sample pH was adjusted to 2 by adding 1 *M* HCl (250 μl) to serum and 0.2 *M* HCl (250 μl) to urine. The sample was extracted with ethyl acetate (2 × 3 ml), and the combined extracts were evaporated under nitrogen at 37°. The residue in the tube was dissolved in 50 μl of methanol and transferred quantitatively, using a 100-μl syringe, to the origin of the thin-layer plate. The tube was rinsed with methanol (50 μl), and the rinsings also were applied to the respective spot.

The plate was developed for approximately 15 cm in ether, dried at air temperature, and redeveloped again for 15 cm in chloroform–methanol–acetic acid (60:40:5). The drug traveled with an R_f of approximately 0.3, and the drug spot was measured on the densitometer. The area of the peak on the chart recorder was calculated for each spot as the product of peak height and width at half peak height.

Standard Curve—A standard calibration curve was prepared daily for each batch of samples. Aliquots of serum or urine (1.0 ml diluted 0–500-fold) spiked with a range of drug concentrations were processed according to the described method, and peak area was plotted against drug concentration. The standard curve was linear over the 1–8-μg/ml range.

Accuracy and Precision—Control serum samples were spiked with unknown quantities of I. The spiked concentrations and concentrations found (in parentheses) were 6.8 (6.8), 1.4 (1.6), 4.1 (3.8), 8.2 (8.2), 2.7 (2.9), and 5.4 (5.5) μg/ml. Control urine samples spiked with unknown quantities of I also were processed, and the results were 4.2 (4.4), 6.3 (6.2), 2.1 (2.3), 7.4 (7.7), and 3.2 (3.2) μg/ml. Six replicate assays were carried out on serum spiked with I at 4.0 μg/ml. The mean value was 4.1 ± 0.1 (*SD*) μg/ml.

DISCUSSION

The described method gives an accurate means of determining the cefazolin concentration in serum and urine and has been used to study its pharmacokinetics in humans. The homogeneity of the spots corresponding to I in extracts of the serum or urine of subjects given I was established by two-dimensional TLC in chloroform–methanol–acetic acid (60:40:5, R_f 0.3) and 2-propanol–water–acetic acid (28:8:1, R_f 0.5). No metabolites were detected in either system. Data from three subjects

[1] Analar.
[2] General-purpose reagent.
[3] Kieselgel $F60_{254}$, E. Merck, Darmstadt, Germany.
[4] TLC Multi-spotter, Analytical Instrument Specialities, Libertyville, Ill.
[5] TLD 100, Vitatron, Dieren, Holland.

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