ACTA CHEMICA SCANDINAVICA 13 (1959) 2039–2046

The Position of the Primary Amino Group in the Steroidal Alkaloid Solanocapsine

PER M. BOLL* and HANS A. LILLEVIK

The Kedzie Chemical Laboratory, Michigan State University, East Lansing, Michigan, U.S.A.

Even under conditions preventing hydrolysis of a possible glycosidic linkage in the steroidal alkaloid solanocapsine, the compound could not be isolated as a glycoside. Data suggesting that the compound has a 3α-amino group instead of the 3α-hydroxyl group usually present in *Solanum* alkaloids are presented. Based on the behavior of acetyl derivatives the hydroxyl group of solanocapsine hitherto considered unreactive is found reactive.

This work was started with the purpose of isolating the antibacterial principle from *Solanum pseudocapsicum*. *In vitro* activity against *Mycobacterium tuberculosis* of root extracts had been reported previously [1]. During the isolation work the antibacterial principle was also found to be present in leaves and stems and was identified as the alkaloid solanocapsine [2].

In 1936 Barger and Fraenkel-Conrat [3] reported the isolation of solanocapsine from *S. pseudocapsicum* and assigned the empirical formula $C_{26}H_{44}N_2O_2$ or $C_{25}H_{42}N_2O_2$ to the compound. Furthermore, the two workers obtained an amorphous alkaloid, solanocapsidine, which was found to have the formula $C_{26}H_{42}N_2O_4$. No alkaloidal glycoside was isolated.

In 1945 Rochelmeyer *et al.*[4] suggested a structural formula for solanocapsine with 27 carbon atoms. The formula was proposed without any experimental evidence.

Later, Schlittler and Uehlinger [5] found that the empirical formula $C_{27}H_{46}N_2O_2$, H_2O was more in agreement with elementary analyses of solanocapsine. By this formula solanocapsine can be classified together with other 27 carbon atom *Solanum* alkaloids (other being solanidine, tomatidine, solasodine *etc.*).

Solanocapsine is the only alkaloid from the genus *Solanum* which has not been isolated as a glycoside. So far, both Barger and Fraenkel-Conrat and Schlittler and Uehlinger have isolated the alkaloid from dried leaf material and have not been able to isolate a glycoside, although the former authors

* Present adress: Royal Danish School of Pharmacy, Chemical Laboratory B, Copenhagen, Denmark.

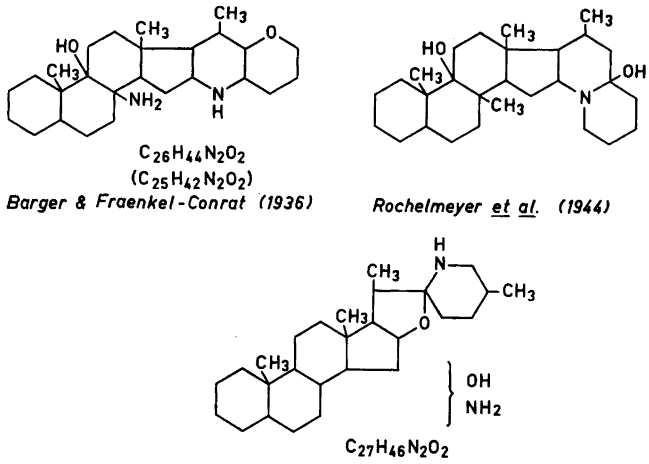

Fig. 1.

obtained reactions for carbohydrates on crude alkaloid preparations, an observation we have confirmed. Therefore, the possibility of an extraordinary labile glycosidic bond being acted upon enzymatically during drying of the plant or during isolation was carefully examined. Enzyme inactivation by placing the plants, immediately after harvesting, in absolute ethanol or in boiling water and using weak acids in the isolation procedure did not yield a glycoside. It is regarded that solanocapsine is present in the plant as a free alkaloid.

It has been stated that *Solanum pseudocapsicum* besides solanocapsine contains an amorphous alkaloid solanocapsidine [3], solanine [6], and solanidine [6]. During the isolation and purification of solanocapsine an amorphous, resinous material was often obtained; but it was always possible, as Schlittler and Uehlinger also observed, by repeated precipitation from aqueous ethanol to convert this material to crystalline solanocapsine. By paper chromatography only one alkaloid was observed, although it has to be mentioned that Schreiber [7] also by paper chromatography has detected another minor alkaloid.

The elementary analysis of solanocapsine was in agreement with the empirical formula proposed by Schlittler and Uehlinger. Since both Barger and Fraenkel-Conrat and Schlittler and Uehlinger have isolated 3′-methyl*cyclo*pentenophenanthrene and 2-ethyl-5-methylpyridine after selenium dehydrogenation of solanocapsine, we have assumed that solanocapsine has a solasodan nucleus and have mainly been interested in the position of the functional groups.

The two above mentioned groups have found that solanocapsine contains a primary amino group, a secondary amino group and a hydroxyl group. The evidence for two amino groups came from methylation of the nitrogen atoms with formic acid and formaldehyde, which gave a trimethyl derivative $C_{30}H_{52}N_2O_2$. This observation was confirmed by the present investigation.

Further evidence for the existence of the two amino groups was given by Schlittler and Uehlinger by reacting solanocapsine with nitrous acid. They observed that by reacting with two equivalents of nitric acid, a neutral, unsaturated nitroso derivative $C_{27}H_{42}N_2O_3$ resulted. In this study the same was observed and a Van Slyke amino nitrogen determination indicated that one mole of nitrogen was liberated per mole of solanocapsine, from which can be concluded that a primary amino group is present. The ultraviolet spectrum of the obtained nitroso derivative showed a peak at 346 mμ and another of

Table 1.

Compound	Maxima	
Nitroso derivative of solanocapsine	346 mμ (log E 2.05)	240 mμ (log E 3.89)
Di(*cyclo*hexylmethyl)-N-nitrosamine [8]	355 mμ (log E 1.95)	240 mμ (log E 3.90)
1-Nitrosopiperidine [8]	350 mμ (log E 2.00)	235 mμ (log E 4.25)
N-Nitrososolasodine [9]	370 mμ (log E 1.98)	234.5 mμ (log E 3.78)

low intensity at 240 mμ. In Table 1 this spectrum is compared with spectra of three nitrosamines. Solanocapsine therefore contains a secondary amino group.

Solanocapsine does not give any precipitate with digitonin contrary to the other *Solanum* alkaloids and is, therefore, not considered to have a 3β-hydroxyl group. It can not have a 3α-hydroxyl group, since it would have been possible to dehydrate such a group. Barger and Fraenkel-Conrat maintain that they could eliminate the hydroxyl group with boiling alkali, but neither Schlittler and Uehlinger nor our investigation could repeat it. On the other hand, the nitroso derivative of solanocapsine gives a precipitate with digitonin. Therefore, it seems plausible to conclude that the primary amino group is placed in the 3-position.

Not only 3β-hydroxy steroids (with 3-OH and 10-CH_3 *cis* to each other) are precipitated as digitonides, but several 3β-amino steroids give precipitates contrary to the corresponding 3α-amino steroids. It is, therefore, assumed that the amino group in solanocapsine is in the 3α-position. An inversion of configuration must have happened during the deamination. Mills [10] described deamination of aminodecalins with nitrous acid and found it to be a true stereospecific reaction. Equatorial amino groups afforded alcohols of same configuration, while axial amino groups reacted by inversion of configuration. That is in agreement with the concept that the 3β-hydroxy group in steroids with the same stereochemical configuration as cholesterol is equatorial and, therefore, thermodynamically more stable.

Hydrogenation of solanocapsine at room temperature and about 3 atm pressure with reduced platinum oxide catalyst gave a derivative, which did not show any melting point depression mixed with solanocapsine, but the

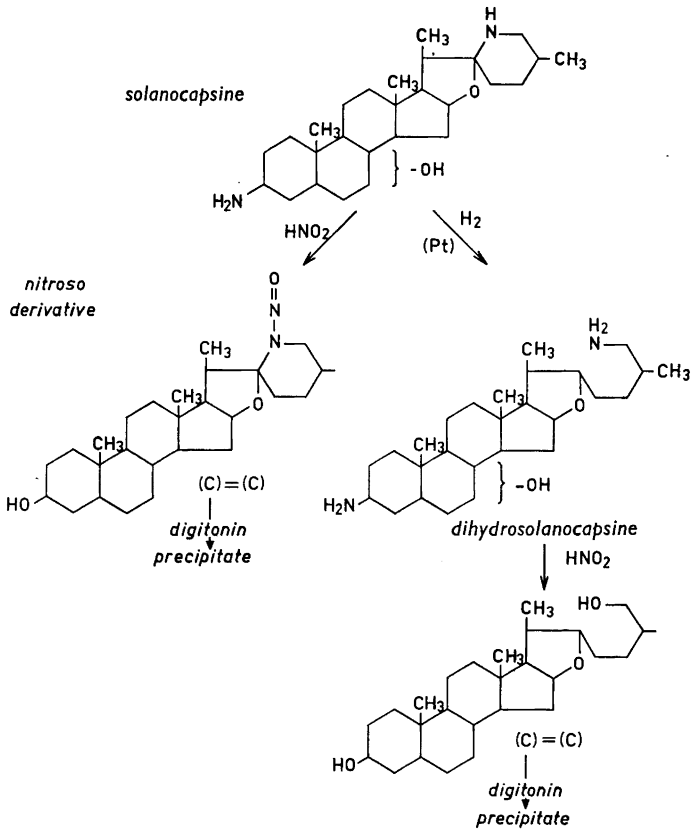

Fig. 2.

specific rotation was changed. A Van Slyke amino nitrogen analysis of the dihydro compound showed that two moles of nitrogen were liberated per mole of compound and that the resulting compound did not give the ultraviolet absorption characteristic for a nitrosamine. It seems safe to conclude that an opening of the piperidine ring has occurred. This is contrary to the opening of the oxide ring in solasodine during hydrogenation.

Schlittler and Uehlinger prepared an amorphous acetyl derivative of solanocapsine, considered by them to be N,N-diacetylsolanocapsine. Several attempts to prepare a diacetate after their procedure never gave the exact elementary analysis expected for a diacetylsolanocapsine. But the compound is not a N,N-diacetylsolanocapsine, but rather an O,N-diacetylsolanocapsine. The reasons for such a conclusion are the following: 1) The derivative was soluble in dilute acetic acid indicating that a basic group probably is present. 2) The infrared spectrum of the derivative showed the characteristic absorption bands for an O-acetyl group and a secondary amide.

Barger and Fraenkel-Conrat acetylated solanocapsine by refluxing with acetic anhydride for 5 h and obtained an amorphous acetate. On the basis of the elementary analysis it was considered to be a diacetate. But it seems that the authors based their calculation on the wrong empirical formula. When recalculated on the basis of a solanocapsine with 27 carbon atoms, the analysis is more in agreement with a triacetate. The acetate prepared in this investigation after their procedure gave an elementary analysis which is in agreement with a triacetate and acetyl group analysis also indicated three acetyl groups. The infrared spectrum showed the bands characteristic for an O-acetyl group and a secondary amide. Furthermore, the intensity of the 6.06 μ band was considerably higher than that of the 6.45 μ band. Since a tertiary amide ($CH_3CO-NRR'$) only shows one characteristic absorption band at 6.06 μ, it is concluded that the third acetyl group is attached to the secondary amino group. The derivative is considered to be O,N,N'-triacetylsolanocapsine.

Barger's as well as Schlittler's group considered the hydroxyl group to be a tertiary or an unreactive hydroxyl group. The conclusion was based on the acetyl derivatives. This investigation has shown that the hydroxyl group can be acetylated, but not tosylated.

EXPERIMENTAL

Isolation. Solanocapsine was isolated and purified by the method of Schlittler and Uehlinger [3]. The plants were grown in the fields belonging to the Department of Horticulture, harvested late fall, and dried in a forced draft oven at 60°. As an example of obtained yields: 500 g of ground leaves gave 8.0 g crude solanocapsine dihydrochloride (1.6 %).

Attemps to isolate an alkaloidal glycoside. Two methods of enzyme inactivation were utilized: 1) Submersion of the harvested plants in absolute ethanol and 2) immediate freezing in dry ice and storage in a deep-freeze until the day of isolation when they were boiled with water.

1) The roots and tops of 15 fresh plants were immediately separated and each part covered with absolute ethanol and stored until time for isolation. A total of 1 400 g of dehydrated tops were macerated in a Waring blender with 14 l of 2 % acetic acid and allowed to stand for 48 h with occasional stirring. The supernatant was removed by filtration and the plant pulp was similarly re-extracted with 5 l of 2 % acetic acid for 24 h and filtered. The combined filtrates were made distinctly alkaline with ammonia and heated to about 50°. After standing overnight the supernatant was decanted and the precipitate collected on a filter. After drying at room temperature the precipitate weighed 0.76 g. The dark brown precipitate was refluxed five times with 100-ml portions of methanol, each time for an hour. 61 mg of residue was obtained. The roots (350 g) were treated in the same manner as described for the tops. After removal of the methanol 20 mg of green residue remained.

2) For the second part of the experiment 13 plants were harvested, immediately placed in dry ice and preserved in the deep-freeze. At the time of glycoside extraction the plants were placed in boiling water for 10 min and then isolation procedure continued in the same manner as above. 128 mg of residue was obtained.

After recrystallization from ethanol the three residues isolated all melted in the broad range of 175—195°C, which suggested impurities. Chromatography in three different solvents showed that the three residues all gave same R_F-value as solanocapsine, when the spots were developed with Dragendorff reagent. If the compounds were chromatographed in 1-butanol-ethanol-water (80:20:20) and the spots developed with aniline hydrogen phthalate, only the compound isolated from the plant tops and the compound isolated from the frozen plants showed spots of D-glucose.

Solanocapsine. To a solution of 500 mg solanocapsine dihydrochloride in 10 ml ethanol-water (1:1) was under stirring added dilute ammonium hydroxide. The yield of crude solanocapsine was 370 mg. After recrystallization from ethanol-water (1:1) it crystallized as platelets which melted with decomposition at 215—216° (lit.[5] m. p. 216— 217°, lit.[3] m. p. 222°); $[\alpha]_D^{25}$ +25 (methanol). The m. p. was usually lower. Only in one case was it possible to reproduce the given m. p. (Found: N 6.20, 6.29; H_2O 3.98; m.wt. 456, 481 (Rast); 482 (neutr. equiv.). Calc. for $C_{27}H_{46}N_2O_2$, H_2O: N 6.24; H_2O 4.01; m.wt. 448.7.)

A Van Slyke amino nitrogen determination gave evolution of 0.99 mole of nitrogen.

No unsaturation and no absorption in the ultraviolet part of the spectrum was observed.

To a solution of 10 mg solanocapsine in 10 ml of ethanol was added 10 ml of a 1 % ethanolic solution of digitonin. The solution remained clear, even after 10 h of standing.

No consumption of periodic acid was observed, even after 10 h. The oxidation was attempted at 3 different hydrogen ion concentrations.

Solanocapsine dihydrochloride. 200 mg of crude solanocapsine dihydrochloride was recrystallized twice from water. The yield was 105 mg and the substance did not melt below 300° (lit.[5] m. p. 300°, lit.[3] m. p. 324°). (Found: C 62.27; H 9.40; N 5.59; H_2O 3.34. Calc. for $C_{27}H_{46}N_2O_2$, 2 HCl, H_2O: C 62.17; H 9.66; N 5.37; H_2O 3.36.)

Solanocapsine picrate. The picrate was prepared as described by Schlittler and Uehlinger. The derivative melted at 198—199° (lit.[3] m. p. 200—201°).

Solanocapsine oxalate. Again the procedure of Schlittler and Uehlinger was followed; m. p. 287—289° (lit.[5] m. p. 288—289°).

Trimethylsolanocapsine. The derivative was prepared following the procedure of Schlittler and Uehlinger. Recrystallized from absolute methanol the compound melted at 208° (lit.[5] m. p. 209°). (Found: N 5.86, 5.72. Calc. for $C_{30}H_{52}N_2O_2$: N 5.93.)

Nitroso derivative. The derivative was prepared according to Schlittler and Uehlinger. After recrystallization from absolute methanol the derivative melted at 194—196° (lit.[3] m. p. 194°, lit.[2] 200°). Microhydrogenation (PtO_2): 0.92 double bond.

Digitonide of nitroso derivative. To a solution of 70 mg of the nitroso derivative in 20 ml ethanol was added 20 ml of a 1 % ethanolic digitonin solution. After standing overnight the copious precipitate was filtered, washed with water and dried to give 45 mg of digitonide. The derivative was washed with 5 ml of ethanol and dried. (Found: N 1.82; 1.90. Calc. for $C_{83}H_{134}N_2O_{32}$: N 1.68.)

Dihydrosolanocapsine. A mixture of 200 mg solanocapsine and 200 mg of platinum oxide in 10 ml of glacial acetic acid and 5 ml of ethanol was shaken under 3 atm pressure in an Adams hydrogen apparatus. After filtration of the catalyst the solvent was removed by distillation under diminished pressure. The residue was dissolved in ethanol-water (1:1) and dilute ammonium hydroxide was added to precipitate 170 mg of compound. It was recrystallized from ethanol-water (1:1), melted at 210—212°, and a mixed melting point with solanocapsine did not show any depression. $[\alpha]_D^{25}$ +40 (methanol). Determination of active "H": Found: 0.42 (room temp.), 0.84 (100°). Calc. for 2 active "H": 0.44, for 4 active "H": 0.89.

A Van Slyke amino nitrogen determination gave evolution of 1.97 moles of nitrogen.

Diacetylsolanocapsine. The derivative was prepared using the procedure of Schlittler and Uehlinger. 150 mg solanocapsine gave 150 mg of a gelatinous acetate. The acetylated alkaloid was chromatographed on 8.7 g of alumina. Eluate fractions of 20 ml were collected. The composition of the fractions is given in Table 2.

Fractions 10—13 were combined and re-precipitated three times from methanol-ethyl ether (1:5). It was not possible to obtain a crystalline product. The amorphous derivative after drying melted at 198—200° (lit.[3] m. p. 193—196°). The compound is soluble in dilute acetic acid. (Found: C 69.90; H 9.84; CH_3CO 1.3 eq, 1.6 eq. Calc. for $C_{31}H_{50}N_2O_4$, H_2O: C 70.77; H 9.62; CH_3CO 2 eq.)

Triacetylsolanocapsine. Barger and Fraenkel-Conrat's procedure was followed. A mixture of 500 mg solanocapsine and 10 ml of acetic anhydride was refluxed in an oil bath for 5 h. The solvent was removed under diminished pressure and the brown, oily residue dissolved in ethanol. Water was added to slight turbidity and the compound was allowed to crystallize. Thus a yield of 400 mg of product melting at 140—150° was obtained. The compound was amorphous and even repeated solution in hot benzene-ligroin

Table 2.

Fraction	Eluant	Residue
1— 3	benzene	9 mg brown oil, soluble in ether
4— 5	benzene + 5 % chloroform	7 mg colorless oil, soluble in ether
6— 7	benzene + 10 % chloroform	9 mg colorless oil, soluble in ether
8— 9	benzene + 25 % chloroform	5 mg colorless oil, soluble in ether
10—13	chloroform + 1 % methanol	79 mg white foam, insoluble in ether
14—16	chloroform + 2 % methanol	6 mg greenish oil, insoluble in ether

did not change it to a crystalline compound. The melting point of the purified compound was 150—155° (lit.[2] m. p. 150—160°).

A further attempt was made to convert the derivative into a crystalline compound by column chromatography. A solution of 83 mg of acetylated solanocapsine in 5 ml benzene was chromatographed on 9 g of alumina and eluate fractions of 20 ml were collected. The composition of the fractions is shown in Table 3.

Table 3.

Fraction	Eluant	Residue
1— 3	benzene	5 mg brownish oil, soluble in ether
4— 5	benzene + 5 % chloroform	4 mg colorless oil, soluble in ether
6— 7	benzene + 10 % chloroform	6 mg colorless oil, soluble in ether
8— 9	benzene + 25 % chloroform	4 mg colorless oil, soluble in ether
10—13	chloroform + 1 % methanol	56 mg white foam, insoluble in ether
14—16	chloroform + 2 % methanol	5 mg greenish oil, insoluble in ether

Fractions 10—13 were combined and after evaporation of solvent gave an amorphous product still melting at 150—155°. The derivative was insoluble in dilute acetic acid. (Found: C 71.61; H 9.49; CH_3CO 2.8 eq. Calc. for $C_{33}H_{52}N_2O_5$: C 71.17, H 9.42; CH_3CO 3 eq.)

Attempted tosylation. A solution of 100 mg solanocapsine in 2.5 ml of pyridine was treated with 100 mg of *p*-toluene sulfonyl chloride. After standing 12 h, 5 ml of water was added and the resulting precipitate was washed with much water. The product was recrystallized from ethanol-water (1:1). Only solanocapsine was recovered as evidenced by mixed melting point and infrared spectrum.

Attempted dehydration. By acid catalysis: A solution of 50 mg of solanocapsine in 15 ml concentrated hydrochloric acid and 35 ml ethanol was refluxed for 10 h. After 50 ml of water was added, the solution was made distinctly ammoniacal and then extracted four times with 100-ml portions of ethyl ether. The combined fractions were dried over anhydrous sodium sulfate and evaporated to dryness. The residue was recrystallized from ethanol-water (1:1). Only solanocapsine was recovered as evidenced by mixed melting point and infrared spectrum. *By base catalysis:* Dehydration in alkaline solution was attempted using the procedure of Barger and Fraenkel-Conrat. The product obtained was found by mixed melting point and infrared spectrum to be solanocapsine. The product gave a condensation product with acetone, which is contrary to the compound thus obtained by Barger and Fraenkel-Conrat.

This investigation was supported in part by research grant E-227 from the *National Institutes of Health.* We wish to thank Professors J. L. Faierley and J. C. Speck of this laboratory for valuable advice.

REFERENCES

1. Frisbey, A., Roberts, J. M., Jennings, J. C., Gottshall, R. Y. and Lucas, E. H. *Mich. Agr. Expt. Sta. Quart. Bull.* **35** (1953) 392.
2. Boll, P. M., Lillevik, H. A., Gottshall, R. Y. and Lucas, E. H. *Antibiotics Ann.* **1955–1956** 255.
3. Barger, G. and Fraenkel-Conrat, H. L. *J. Chem. Soc.* **1936** 1537.
4. Rochelmeyer, H., Stützel, H. and Chen, H. *Arch. Pharm.* **282** (1944) 92.
5. Schlittler, E. and Uehlinger, H. *Helv. Chim. Acta* **35** (1952) 2034.
6. Muenscher, W. C. *Poisonous Plants of the United States*, The Macmillan Company, New York 1951, p. 210.
7. Schreiber, K. *Personal communication.*
8. Goldberg, M. V. and Kirchsteiner, H. *Helv. Chim. Acta* **26** (1943) 289.
9. Briggs, L. H., Harvey, W. E., Locker, R. H., McGillivray, W. A. and Seelye, R. N. *J. Chem. Soc.* **1950** 3013.
10. Mills, J. A. *J. Chem. Soc.* **1953** 260.

Received July 6, 1959.