Studies in the Chemical Constituents of the Fresh Berries of *Solanum xanthocarpum* Schrad. And Wendle.

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Summary: Chemical investigations of the fresh, undried, ripe and unripe berries of *Solanum xanthocarpum* collected from the arid areas of Pakistan have led to the isolation of three glyco bases: solasonine, solamargine and solasurine, apart from an apparently partially hydrolysed glyco base. The solasodine content was noted as 0.75% and 1.7% (on dry weight basis) in ripe and unripe berries, respectively. From the non-alkaloidal constituents, the galactoside of β-sitosterol and two phenolic substances identified as the methyl ester of 3,4-dihydroxycinnamic acid (methyl caffeate) and 3,4-dihydroxycinnamic acid (caffeic acid) have been isolated.

Introduction

Solanum xanthocarpum, Schrad. and Wendle. is a prickly plant which grows wild in different regions of the Indo-Pakistan sub-continent. Various medicinal properties are attributed to it, particularly in the treatment of asthma, chronic cough and catarrhal fever[1]. Chemical examination of its berries by Saiyed and Kanga in 1936[2], led to the isolation of a glycoalkaloid, solasonine, obtained earlier from Solanum sodomeum Linn[3]. Subsequently, Seth and Chatterjee[4] communicated the isolation of two further glycoalkaloids: solamargine and solasurine, apart from solasonine, through chromatographic technique from *Solanum surattense* Burm. f. (syn: *Solanum xanthocarpum*[5,6]. The hydrolysis of these glycoalkaloids yielded in each case solasodine as the aglycone, along with its dehydro base which might be an artifact.

The solasodine content of the berries of S. xanthocarpum is reported to vary from 1.1% to 4.6%[7,8], depending apparently on climatic and soil conditions. Taking into account the therapeutic importance of solasodine and its derivatives, it was considered of interest to investigate the fresh, undried *S. xanthocarpum* berries collected from the arid areas of Pakistan. As a result of these studies using classical procedures of isolation, three glycoalkaloids solasonine, solamargine and solasurine have been obtained from ripe and unripe berries in good yields along with a partially hydrolysed glycoalkaloid. Furthermore, solasurine, the isolation of which is reported in the literature[4] through thin layer chromatography, has been obtained in sizable quantity through solvation in methanol/alcohol and fractional precipitation with water, as described in the experimental.

It has been observed that berries collected in autumn (Sept.Oct.) yielded only solasonine and solamargine without any trace of solasurine which was obtained from the material collected in summer (May–June). The solasodine content of unripe berries was 1.7% (on dry weight basis) as against 0.75% noted for the ripe berries.

From the non-alkaloidal portion a glycoside of β-sitosterol with galactose as the sugar moiety has been obtained along with two phenolic substances, which could be identified as methyl caffeate and caffeic acid, the occurrence of which in *S. xanthocarpum* had not been noted earlier.

Experimental

Melting points were recorded in glass capillary tubes and are uncorrected. IR and UV spectra were measured with SP 200 G and SP 800 spectrophotometer respectively. Proton NMR spectrum was recorded on Jeol PMX 60 instrument with TMS as internal reference. Mass spectra were recorded on MAT 112 S and Varian MAT 312 spectrometer. The purity of the samples was checked on tlc/silica-gel.

Twenty kg fresh ripe berries of *S. xanthocarpum* collected in the month of June were soaked in alcohol without drying or crushing, and percolated five times with the solvent at intervals of 48 hrs. The extracts were concentrated in vacuo below 50°C to a thick liquidish consistency. The concentrate was divided up into water soluble (A), ethyl acetate soluble (B), and ethyl acetate/water insoluble (C) fractions. The aqueous solution (A) was basified with ammonia to pH 7.5–8 and the liberated base filtered, washed with water and

dried on porous plate. The crude base (2% on dry weight basis) was taken up in dilute acetic acid and pH of the solution adjusted to 6.5 with addition of ammonia. A negligible quantity of darkish precipitate was filtered off and the filtrate was charcoaled. The base obtained from the nearly colourless solution on liberation with ammonia was divided into alcohol soluble and insoluble portions. The latter fraction was taken up in acetone and filtered from a little insoluble material. On keeping the concentrated acetone solution in cold a white crystallizate settled down, which on recrystallization from the same solvent gave a single spot on tlc. It melted at 295-300° (decomp.) and showed no depression of m.p. on admixture with the pure sample of solamargine (0.3% on dry weight basis). The alcohol soluble fraction was freed of solvent and taken up in 50% alcohol. The solution was concentrated on the waterbath in a crystallizing dish when on subsequent slow evaporation at room temperature, a crystalline mass was obtained which was filtered, washed with cold 50% alcohol and divided up into methanol soluble and methanol insoluble fractions. The methanol soluble portion, on repeated crystallizations from the same solvent, ultimately yielded fine needles of solasurine m.p. 246-8° (0.4% on dry weight basis). The methanol insoluble base gave slender rods of solasonine m.p. 300-10° (decomp.) on recrystallization from 90% methanol (0.7% on dry weight basis).

All the filtrates from the above working containing two or three spots were taken together and subjected to hydrolysis.

Hydrolysis of Glycoalkaloids : 10g of the glyco bases were hydrolyzed with 150 ml of 2.4 N aqueous hydrochloric acid on the boiling water bath for 45 minutes. The hydrochloride of the hydrolyzed base which crystallised out on cooling the reaction mixture, was filtered, washed with cold water and treated with dilute ammonia. The liberated base was filtered, washed and dried on porous plate. It was then digested out with petroleum ether followed by a mixture of 9:1 benzene-methanol. On concentration and keeping in cold, both the digestates yielded fine needles of solasodine (Total 4g; 40% on the weight of the total glyco bases), which was identified through comparison on tlc and mixed m.p. with an authentic sample, i r, mass and ^{1}Hnmr spectra . The mother liquor of solasodine from the petroleum ether soluble fraction yielded a dehydro base as flowers of fine needles m.p. 169-70° (0.01g; 0.1% on the weight of total glyco bases). This was identified as solasodiene (lit. m.p. 173°)[9] through spectral data.

UVλ_{max} (hexane):244 (log ϵ=4.152), 235 (log ϵ= 4.336) shoulder at 228(nm).

IR ν_{max} (KBr) (cm^{-1}): 1600,1650,2840,3350.

Mass: (m/e): M^+ at 395, other important peaks are at 380, 282, 138 (base peak), 114.

This compound is apparently an artifact formed from solasodine through dehydration during acidic hydrolysis of the glyco bases.

The small quantity of benzene-methanol insoluble fraction showed one spot on tlc which did not correspond to either of the glyco bases or to solasodine. On crystallization from methanol it formed rods m.p. 250-60°. This on further hydrolysis with 2.4 N aqueous hydrochloric acid for half hour yielded a further quantity of solasodine and sugars showing two spots on paper chromatogram identified as glucose and galactose.

The filtrate from the main hydrolyzed base hydrochloride, when subjected to paper chromatography using n-butanol-ethanol-water (4:1:5) as developing solvent, showed the presence of glucose, galactose and rhamnose.

Hydrolysis of pure solasonine, solamargine and solasurine following the procedure noted above yielded the same aglycone, solasodine. The sugar portions of these were identified through paper chromatography as glucose, galactose and rhamnose in solasonine and glucose, rhamnose in solamargine and solasurine as reported in literature[4,10]

More or less the same procedure was adopted for the isolation work on unripe berries and the solasodine content was noted to be 1.7% (on dry weight basis) as against 0.75 in ripe berries,

The ethyl acetate soluble fraction (B) was concentrated under reduced pressure to a small volume and charcoaled. The light coloured ethyl acetate solution was extracted out with dilute alkali and the ethyl acetate and aqueous alkaline layers worked up in the usual manner to obtain the acidic and neutral components. On crystallization from methanol-benzene (1:1) the neutral fraction yielded slender shining needles of the glycoside which was soluble in hot methanol and ethanol and insoluble in benzene, ethyl acetate and chloroform and melted at 277-78°C (decomp.), Mass: (m/e): 396 (M^+–$C_6H_{12}O_6$), 381, 329, 303 and 43 (100%); IRν_{max} KBr: 3450 cm^{-1} (OH).

Hydrolysis of the Glycoside: The glycoside was refluxed with 2N alcoholic hydrochloric acid on boiling water bath for 3 hours and the dirty white precipitate that gradually separated out was then filtered, washed and dried on porous plate. It crystallized out from ether in shining fine needles m.p. 140-41°C and gave positive test for sterols. The above filterate which gave positive test for carbohydrates was neutralized with 30% ammonium hydroxide and concentrated under reduced pressure. It was taken in methanol, filtered and subjected to paper chromatography on filter paper Whatman No. 1 in the ascending order along with standard sugars using solvent system n-butanol-ethanol-water (4:1:2.8). Aniline hydrogen phthalate[11] was used for developing the spots. A single brown spot appeared corresponding to galactose.

Charaterization of the Aglycone: IR $\nu_{max.}$ (KBr): 1070-1080 cm^{-1} (C–OH st.), 2850-2990 cm^{-1} (C-C-H and C = C-H st.), and 3400-3500 cm^{-1} (OH st.).

Mass m/e: 414 (M^+), 399 = $(M\text{-}CH_3)^+$, 396 = $(M\text{-}H_2O)^+$, 381 = $(M\text{-}CH_3\text{-}H_2O)^+$; other important fragments at m/e 329, 303, 275. 288, 273, 253 and 43 (100%). The aglycone was identified as β-sitosterol from the data noted above and mixed m.p. with an authentic sample.

The alkaloid free aqueous solution of the main alcoholic extract was hydrolysed with 1N aqueous HCl for 3 hours on boiling water bath. It was worked up in the usual manner and divided into neutral and acidic fractions. The benzene soluble fraction of the latter finally yielded on repeated crystallizations from ether, slender rods which melted at 158-9° (A). Similarly the benzene insoluble portion of it yielded an acidic product which formed colourless shining needles on crystallizations from acetone, m.p. 199-200°C.

Characterization of A: The molecular formula of A was found to be $C_{10}H_{10}O_4$ through high resolution mass spectroscopy. Mass spectrum showed M^+ at m/e 194.05991 (93.4%); other important fragments were observed at m/e 163 (100%), 146, 136, 135, 134, 105, 89, 77, 67, 63, 55, 53; IR $\nu_{max.}$ (KBr) cm^{-1}: 1050 (C–OH), 1460, 1550, 1600 (benzene ring), 1620 (C = C) 1690 (carbonyl), 3500 (OH); UV λ_{max} (methanol): 330 (log E = 4.315) 299 (log E = 4.225), 245 (log E = 4.111), 220 (log E = 4.242), 208 (log E = 4.287) (nm).

The presence of two hydroxyl groups was confirmed through diacetate (acetic anhydride/pyridine) m.p. 118-20°C, M^+ 278 and dimethyl (diazomethane) m.p. 62-4°C, M^+ 222.0886 derivatives.

The above physical and chemical data led to the suggestion that the phenol contains two hydroxyl groups and one ∝, β-unsaturated methyl ester functions. The position of these were ascertained through ^{1}Hnmr (DMSO-d_6) spectrum, which showed the chemical shifts (δ) at 3.8 (3H, $-CO_2Me$), 6.23 (1H, J = 16 Hz) ($-C{=}CH-CO_2Me$), 7.5 (1H, J=16 Hz $-CH{=}CH-CO_2Me$) 6.93 (1H, C_2-H), 6.73 (1H, $J_{5\,6}$ = 8Hz, C_5-H), 6.9 (1H, $J_{6\,5}$ = 8Hz, C_6-H), 7.1 broad two protons signal (2-OH).

Furthermore its alkaline hydrolysis (6% methanolic alkali) gave caffeic acid m.p. 198-200°C, M^+ at m/e 180. The physical and chemical data noted above established its identity as methyl caffeate[12].

Characterization of B: Mass: M^+ at m/e 180, other prominent peaks are at 162 $(M-H_2O)^+$, 135 $(M-COOH)^+$, 136 $(M-CO_2)^+$, 119, 110, 107, 77, 72, 73, 59, 83, 44 (100%) and 43 m/e.

IR $\nu_{max.}$ (KBr): 2500-3400, 770, 795, 810, 850, 970, 1120, 1020, 1510, 1620, 1670 cm^{-1}.

The melting point and spectral data of the product and mixed melting point and tlc with the acid obtained on the hydrolysis of methyl caffeate served to establish its identity as caffeic acid[12].

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