Alkaloidal Glycosides from *Solanum dulcamara* V *. The Constitution of α-Solamarine

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In a previous paper [1] the isolation of three new alkaloidal glycosides from *Solanum dulcamara* L. was described, and the constitution of two of these, β- and γ-solamarine, was recently established in this laboratory.[2] The aglycone of the third glycoside, α-solamarine, has already been shown to be Δ^5-tomatidenol-(3β) and it has also been established that this glycoside on hydrolysis yields one mole each of galactose, glucose and rhamnose.[1] The carbohydrate moiety of α-solamarine has now been investigated further and shown to be solatriose, a branched trisaccharide also found as the carbohydrate moiety of α-solanine [3] and solasonine.[4]

On hydrolysis, α-solanine and solasonine,[6] as well as α-solamarine yield one mole each of the same monosaccharides. Kuhn *et al.*[7] have subjected solasonine to periodate oxidation and after hydrolysis of the product, detected galactose chromatographically as the only surviving sugar, a result also obtained with α-solamarine, thus indicating a branched glycoside for both compounds. Partial hydrolysis of α-solamarine with 0.01 N hydrochloric acid for 150 min afforded besides galactose, glucose and rhamnose two oligosaccharides. The two oligosaccharides had $R_{lactose}$-values in agreement with those of solatriose [3] (Fig. 1. R=H) and solabiose (3-*O*-β-D-glucopyranosyl-D-galactopyranose)[3] when chromatographed in the upper layer of butanol-pyridine-water (3:1:1.5), to which is added an equal volume of pyridine [8] (Found: $R_{lactose}$ 1.05 and 1.25. Lit. values [3] 1.08 for solatriose and 1.26 for solabiose). Parallel runs with authentic solatriose gave also an $R_{lactose}$-value of 1.05. Only two oligosaccharides were detected, although partial hydrolysis of a branched alkaloidal trisaccharide might yield three. This observation is in accordance with thin layer chromatography of the alkaloidal products obtained by the above hydrolysis revealing besides unhydrolyzed α-solamarine only three Dragendorff positive spots with R_F-values 0.57, 0.83, and 1.00, the latter being impure and consisting of a mixture of Δ^5-tomatidenol-(3β) and $\Delta^{3,5}$-tomatidadiene (Solvent: Bottom layer of chloroform-ethanol-1 % ammonia (2:2:1)[1]).

Partial acid hydrolysis of α-solamarine gave only a poor yield of oligosaccharides; hence degradation with hydrogen bromide-glacial acetic acid was attempted. Refluxed with acetic anhydride-pyridine for 2 h α-solamarine gave an amorphous deca-acetyl-α-solamarine, m.p. 145–148°, $[\alpha]_D^{27}$ −22.7° (ethanol, *c* 1.28). (Found: C 58.17; H 7.16. Calc. for $C_{65}H_{93}NO_{26}$, 2 H_2O: C 58.18; H 7.30). Degradation of 1.5 g of the peracetate with hydrogen bromide-glacial acetic acid and saponification of the acetate with methanolic ammonia as described by Kuhn *et al.*[3] gave 0.38 g of a fraction containing the monosaccharides and solatriose besides small amounts of solabiose and another oligosaccharide, all detected paperchromatographically. Chromatography on active carbon-Celite (1:1) resulted after elution with water followed by water containing increasing amounts of ethanol in fractions with monosaccharides, solabiose + the unknown oligosaccharide + solatriose, and finally 57 mg of solatriose (eluted with 8 % and 12 % ethanol in water). The fractions with amorphous solatriose could not be induced to crystallize from a mixture of methanol and absolute ethanol before seeding with authentic solatriose followed by partial evaporation of the solvent at room temperature. M.p. alone or in admixture with authentic solatriose ~ 196° (decomp.) after slight foaming at 145–160° (Lit. value [3] 200° and foaming at 150–160°; $R_{lactose}$ 1.05; $[\alpha]_D^{27}$ after 5 min −6.8°; after 60 min −3.9° (*c* of the amorphous product 1.15; lit. value [3] after 5 min −7.5°; after 60 min −4.4°). (Found: C 42.73; H 6.80. Calc. for $C_{18}H_{32}O_{15}$, 1 H_2O: C 42.68; H 6.77). On hydrolysis with N hydrochloric acid paper chromatography revealed galactose, glucose, and rhamnose.

The isolation of solatriose identifies α-solamarine as a solatrioside of Δ^5-tomatidenol-(3β). Using Klyne's rule [9] the molecular rotation ($[M]_D$ 10^{-2}) of methyl-β-solatrioside is calculated as the difference between the molecular rotations of the alkaloidal glycoside and the aglycone for α-solanine, dihydro-α-solanine and sola-

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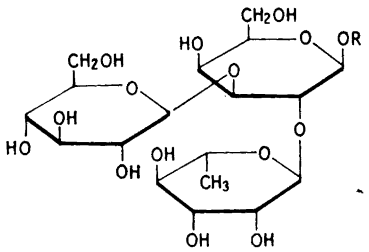
sonine. The values calculated are $-409°$[10], $-476°$[3], $-435°$[7,11], and $-205°$[6] (*cf.* Ref.[12]). For the methyl solatrioside of *a*-solamarine the molecular rotation is $-265°$,[1] a value in fairly good agreement with the molecular rotation of methyl-*β*-solatrioside. Hence the constitution of *a*-solamarine is considered to be *O*-*a*-L-rhamnopyranosyl-(1→2)-*O*-[*β*-D-glucopyranosyl-(1 → 3)]-*β*-D-galactopyranosyl-Δ^5-tomatidenol-(3*β*) (Fig. 1. *R* = Δ^5-tomatidenol-(3*β*)).



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Thin-layer Chromatography of Bile Pigments

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The direct and indirect diazo reaction of the bile pigments was first described by Hijmans van den Bergh and Müller [1] in 1916. Numerous attempts were made after this pioneer work to characterize the bile pigments giving the positive diazo reaction, but the solution to the problem was not provided until 1956, when these pigments were shown to be conjugates of bilirubin and glucuronic acid. This was done by means of partition chromatography and countercurrent distribution [2] or by paper chromatography.[3,4] It was possible to demonstrate by means of partition chromatography [5] or paper chromatography [6] that the direct reacting bilirubin consists of two components, called pigments I and II. The more polar pigment (II) is bilirubin diglucuronide, whereas pigment I is probably bilirubin monoglucuronide. In addition to bilirubin glucuronides, other conjugates of bilirubin which are also polar and water-soluble can occur physiologically. One of these, bilirubin sulphate, has been identified by Isselbacher and McCarthy [7] by means of $^{35}SO_4$ tracer studies.

Only a few quantitative techniques have been described to determine bilirubin and its conjugates. Most of them are also time-consuming and inaccurate. In the present study thin-layer chromatography has been applied to the quantitative determination of bilirubin and its conjugates and to biliverdin.

With a thin-layer applicator (Desaga, Heidelberg), glass plates (76 × 26 × 1 mm) were coated with a well-stirred suspension of Kieselgel G (E. Merck, Darmstadt), 30 g in 60 ml water. The plates were dried at 105—110°C for 30 min and stored in a desiccator until required.

Diazotized human and hen's bile was applied with a micropipette on the starting point 1.0—1.5 cm from the end of the plate after the extraction of bile acids with a 7:3 mixture of heptane and butanol as well as with petro-