sonine. The values calculated are -409^{+10} , -476^{+3} , $-435^{+7,11}$, and -205^{+6} (cf. Ref.¹²). For the methyl solatrioside of α -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 α -solamarine is considered to be *O*- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-*O*-[β -D-glucopyranosyl-(1 $\rightarrow$ 3)]- β -D-galactopyranosyl-*D*⁵-tomatidenol-(3 β) (Fig. 1. *R* = *D*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 Müller¹ 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 distribution² or by paper chromatography.^{3,4} It was possible to demonstrate by means of partition chromatography⁵ or paper chromatography⁶ 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 McCarthy⁷ by means of ³⁵SO₄ 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-

Fig. 1. Separation of bile pigments by thin-layer chromatography on Kieselgel G (for solvent systems A and B see text). (b) Biliverdin; (I) Bilirubin; (II, III) Glucuronide conjugates of bilirubin; (IV) Sulphate conjugate of bilirubin; (s) Start.

leum ether. The composition of the solvent systems were as follows:

A: methylethylketone:propionic acid:water (10:2.5:2.5)

B: butanol:acetone:propionic acid:water (7:4:3:3)

Using solvent system A it was possible to get four diazo-positive fractions, I, II, III, and IV. Fraction I ($R_F = 0.57$) consisted of unconjugated bilirubin. It did not contain any glucuronic acid, and it had the same R_F -value as free bilirubin. Fraction II ($R_F = 0.37$) was equivalent to pigment I of Cole *et al.* and fraction III ($R_F = 0.20$) was bilirubin diglucuronide (pigment II), as concluded from the molar ratios between the glucuronic acid and fractions II and III. Fraction IV ($R_F = 0.09$) is probably the sulphate conjugate of bilirubin. When solvent mixture B was used, the corresponding R_F -values were 0.70, 0.58, 0.43 and 0.20. The R_F -value of biliverdin was in both cases 0.72–0.80. Occasionally thin-layer chromatography revealed, in addition to fractions I–IV, small amounts of other diazo-positive derivatives.

When inorganic $^{35}\text{SO}_4$ (900 μC) was administered intraperitoneally to hens and the bile collected after 3 h, and diazotized and purified as above, two to three stronger radioactive fractions were obtained in autoradio-

graphy. The proportionately most marked of them was equivalent to the "sulphate fraction" (IV).

For the identification of fraction IV synthetic "bilirubin sulphate" was also prepared.⁸ Bilirubin, when treated with sulphuric acid in the Liebermann-Burchard reaction, is converted to a direct-reacting pigment, which is probably a sulphate derivative. This compound had the same R_F -value as fraction IV.

The fractions were then carefully eluted with a methanol-water mixture (1:1 v/v) and centrifuged, and the absorption read at 540 $m\mu$ in a Beckman spectrophotometer. The azo derivatives were subjected then to acid hydrolysis, which was carried out in 1 N hydrochloric acid at 100°C for 90 min. The hydrolysates were decolorized with activated charcoal, centrifuged, and analysed for their content of glucuronic acid.¹⁰ The molar ratios of glucuronic acid to diazo fractions II and III were calculated.

This method allows the determination of bilirubin, its conjugates and biliverdin in minute amounts in both bile and serum. In the latter case the precipitation of lipoproteins is necessary.

A more detailed report of the methods and results described here will be published in the near future.

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