CCLI.—*Syntheses of Glucosides. Part I. The Synthesis of Indican.*

By ALEXANDER ROBERTSON.

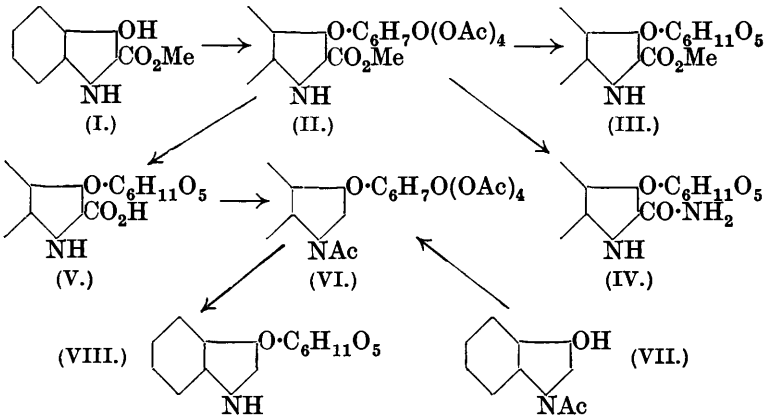
THE glucoside indican, 3-β-glucosidoxyindole, was first isolated by Schunk (*Phil. Mag.*, 1855, **10**, 74; 1858, **15**, 127) from *Polygonum tinctorium*, who stated that the indigotin-yielding substance in *Isatis tinctoria*, "woad," was the same. It was, however, not until many years afterwards that its correct formula was indicated by Marchlewski and Radcliffe (*J. Soc. Chem. Ind.*, 1898, **17**, 434), who suggested that the glucoside had possibly the formula $C_{14}H_{17}O_6N$, and that, on hydrolysis, glucose and indoxyl were formed, the latter on oxidation being converted into indigotin. The proof that indican is an indoxyl glucoside, and that the sugar obtained from it is glucose, was supplied by Hazewinkel (*Proc. K. Akad. Wetensch. Amsterdam*, 1900, **2**, 512). About the same time, Hoogenwerff and ter Meulen (*ibid.*, p. 520) isolated the glucoside in a crystalline condition from *Polygonum tinctorium* and *Indigofera leptostachya*. They showed that it had the formula $C_{14}H_{17}O_6N$ and when crystallised from water contained $3H_2O$. By hydrolysis of the glucoside in the presence of air, they obtained slightly impure indigotin; and later the identity of the sugar with *d*-glucose was indicated by ter Meulen (*Rec. trav. chim.*, 1905, **24**, 444). Beyerinck (*Proc. K. Akad. Wetensch. Amsterdam*, 1900, **3**, 101) showed that the glucoside in *Polygonum tinctorium* is not the same as the indigo-yielding substance in *Isatis tinctoria* as claimed by Schunk, and also that indican was slowly hydrolysed by emulsin, as well as by its specific enzyme, indemulsin.

Perkin and Bloxam (J., 1907, **91**, 1715), Perkin and Thomas (J., 1909, **95**, 793), and Thomas, Bloxam, and Perkin (J., 1909, **95**, 824), working with improved methods, isolated the crystalline glucoside from *Indigofera leptostachya*, *I. sumatrana*, *I. arrecta*, and *Polygonum tinctorium*, and confirmed in detail the work of Hoogenwerff and ter Meulen, Hazewinkel, and Beyerinck. In addition, these workers obtained the glucoside in the anhydrous state. From a study of the hydrolysis of methylated indican, Macbeth and Pryde (J., 1922, **121**, 1660) showed that the glucoside is in all probability a normal β-derivative. This is in agreement with the conclusions arrived at by ter Meulen (*loc. cit.*) as a result of a study of the hydrolysis of the glucoside by enzymes.

In attempting the synthesis of indican, the most obvious method was to obtain the penta-acetyl derivative by the interaction of 1-acetyl-3-hydroxyindole and tetra-acetyl-α-glucosidyl bromide in

acetone solution in the presence of aqueous alkali. Reference to the literature indicated the difficulty of preparing a sufficient quantity of the acetylhydroxyindole, and the instability of the latter in the presence of alkali. Accordingly, another method for the synthesis of the glucoside was devised.

Methyl 3-O-*tetra-acetyl*-β-*glucosidoxyindole*-2-*carboxylate* (II) was obtained in good yield by the interaction of methyl 3-hydroxyindole-2-carboxylate (I) and tetra-acetyl-α-glucosidyl bromide in acetone solution in the presence of potassium hydroxide.



This penta-acetyl glucoside on hydrolysis with methyl-alcoholic potash yielded 3-β-*glucosidoxyindole*-2-*carboxylic acid* (V), which was conveniently isolated from the reaction mixture as the potassium salt. The latter, when treated with fused sodium acetate and acetic anhydride, first on the water-bath and then at 160°, was acetylated and the carboxyl group simultaneously eliminated with the formation of 1-*acetyl*-3-O-*tetra-acetyl*-β-*glucosidoxyindole* (VI). In order to verify the constitution of this substance, a small quantity of it was prepared directly by the interaction of 1-acetyl-3-hydroxyindole (VII) (D.R.-P. 108761) and tetra-acetyl-α-glucosidyl bromide in a cooled acetone solution in the presence of potassium hydroxide. The specimens of *penta-acetylindican* thus obtained were identical. The penta-acetyl glucoside was deacetylated with methyl-alcoholic ammonia, and 3-β-*glucosidoxyindole*, indican (VIII), obtained in good yield. The properties of the synthetic glucoside were identical with those of the natural product described in detail by Perkin and his collaborators (*loc. cit.*).

Hydrolysis of the synthetic indican under various conditions gave the same products as those obtained by these authors, by Hazewinkel (*loc. cit.*) and by Beyerinck (*loc. cit.*).

On deacetylation of the glucoside (II), a mixture of *methyl* 3-β-*glucosidoxyindole*-2-*carboxylate* (III) and 3-β-*glucosidoxyindole*-2-*carboxylamide* (IV) was obtained, but the latter could not be isolated in a crystalline condition. Hydrolysis and acetylation, however, established the nature of the uncrystallised syrup.

The preparation of methyl 3-hydroxyindole-2-carboxylate (Vorländer, *Annalen*, 1898, **301**, 349) was carried out by the method described for the preparation of the corresponding ethyl ester (D.R.-P. 105495). This methyl ester on acetylation yielded an O-*acetyl* derivative.

EXPERIMENTAL.

Methyl 3-*Hydroxyindole*-2-*carboxylate* (I). — To pulverised sodium (2·7 g.) and methyl phenylglycine-*o*-carboxylate (25 g.) in benzene (100 c.c.), one drop of anhydrous methyl alcohol was added and the mixture was gently warmed on the steam-bath until a vigorous reaction set in; the sodium derivative of methyl 3-hydroxyindole-2-carboxylate was then deposited as a solid. When this reaction had almost ceased, the mixture was heated under reflux for 30 minutes and then cooled. The sodium derivative was collected, washed with benzene, dried, and dissolved in ice-water (250 c.c.), and the solution was filtered after treatment with charcoal and acidified with acetic acid (50%). The indoxylic ester thus precipitated crystallised from methyl alcohol (70%) (charcoal) in elongated, glistening needles, m. p. 157—158°. Yield, 15 g.

Methyl 3-*acetoxyindole*-2-*carboxylate* was obtained in almost theoretical yield when methyl 3-hydroxyindole-2-carboxylate (2 g.), fused sodium acetate (2 g.), and acetic anhydride (10 c.c.) were heated under reflux for 1 hour. Water was added to the cooled mixture, and the *acetyl* derivative gradually crystallised. After recrystallisation from methyl alcohol (50%) and then from benzene–ligroin (1 : 9), it was obtained in glistening, elongated prisms, m. p. 145° (Found: C, 62·1; H, 4·7. $C_{12}H_{11}O_4N$ requires C, 61·8; H, 4·7%). It is insoluble in dilute alkali solution. Attempts to obtain a diacetyl derivative were unsuccessful.

Methyl 3-*Tetra-acetyl*-β-*glucosidoxyindole*-2-*carboxylate* (II).—A cooled solution of potassium hydroxide (2·8 g.) in water (20 c.c.) was gradually added to one of methyl 3-hydroxyindole-2-carboxylate (9·3 g.) and tetra-acetyl-α-glucosidyl bromide (20·5 g.) in pure acetone (90 c.c.) cooled to 10°; the acetyl glucoside soon began to crystallise. After remaining at room temperature for 4 hours, the mixture was diluted with water (50 c.c.) and cooled in ice, and the solid was collected and recrystallised from hot methyl alcohol (charcoal). The *acetyl glucoside* was thus obtained in colourless

prisms with blunt ends, m. p. 229—230° (Found: C, 55·5; H, 5·2. $C_{24}H_{27}O_{12}N$ requires C, 55·5; H, 5·2%). Yield, 16·5 g.

This glucoside is sparingly soluble in ether and in cold alcohol, and moderately easily soluble in hot alcohol. A penta-acetyl derivative could not be obtained.

3-β-*Glucosidoxyindole-2-carboxylamide* (IV).—Dry methyl alcohol (200 c.c.) containing finely powdered methyl 3-tetra-acetyl-β-glucosidoxyindole-2-carboxylate (3 g.) in suspension was saturated at 0° with dry ammonia gas. The glucoside gradually dissolved and the solution was allowed to remain at 0° for 14 hours. After removal of the ammonia and methyl alcohol in a vacuum at 18°, the residual oil was freed from acetamide by distillation of the latter at 100°/1—2 mm. The straw-coloured syrup which remained was dissolved in water, and the solution on evaporation in a vacuum desiccator deposited the *amide* as a white solid. This crystallised from water in colourless, rectangular plates, which decomposed at 254—256° to a black mass after darkening at 245° (Found: C, 50·7; H, 5·6; N, 7·5. $C_{15}H_{18}O_7N_2$ requires C, 50·8; H, 5·6; N, 7·8%). Yield, 0·3 g. The amide is fairly soluble in alcohol and in hot water.

The filtrate after the separation of the amide was evaporated to small bulk, and a straw-coloured syrup obtained which could not be induced to crystallise. This undoubtedly consisted of impure *methyl* 3-β-*glucosidoxyindole-2-carboxylate* (III). When this syrup was heated to boiling with 6% hydrochloric acid for 1 minute, methyl 3-hydroxyindole-2-carboxylate (I) crystallised, m. p. 157—158° after recrystallisation from 70% methyl alcohol. The acetyl derivative, m. p. 145°, was identical with that described above. The syrup (1 part) was acetylated by heating it under reflux for 1 hour with fused sodium acetate (1 part) and acetic anhydride (10 parts). Water was added to the cooled mixture to decompose the excess of acetic anhydride, and methyl 3-tetra-acetyl-β-glucosidoxyindole-2-carboxylate separated, m. p. 229—230° after recrystallisation from methyl alcohol. A mixture of the latter with an authentic specimen of the acetylated glucoside showed no depression of the melting point.

3-β-*Glucosidoxyindole-2-carboxylic Acid* (V).—A solution of potassium hydroxide (4 g.) in 80% methyl alcohol (25 c.c.) was gradually added to a suspension of methyl 3-tetra-acetyl-β-glucosidoxyindole-2-carboxylate in methyl alcohol (50 c.c.). After the solid had dissolved, the solution was kept at room temperature for 12 hours, methyl alcohol (20 c.c.) was then added, and the mixture heated under reflux on the water-bath for 2 hours, during which time the potassium salt of 3-β-glucosidoxyindole-2-carboxylic acid crystallised

out. When cold, the colourless potassium salt was collected, washed with dry methyl alcohol, and dried. The salt (yield, 3·5 g.) was sufficiently pure for use in the next stage of the synthesis.

A solution of the potassium salt (1 g.) in water (4 c.c.) was acidified with a slight excess of 2% hydrochloric acid. The pale blue, crystalline precipitate of the *glucoside* which separated was collected and recrystallised from warm water (charcoal), from which it separated in almost colourless, rod-like prisms, which turned brown at 215—220° and decomposed to a dark liquid at 230—231° (Found : C, 53·1; H, 5·1; N, 4·0. $C_{15}H_{17}O_8N$ requires C, 53·1; H, 5·0; N, 4·1%).

A solution of this glucoside in dilute hydrochloric acid, containing ferric chloride, on boiling is quickly decomposed with the formation of indigotin. An aqueous solution when heated to boiling quickly assumes a blue colour, due to slight hydrolysis and the formation of a trace of indigotin.

1-*Acetyl*-3-*tetra-acetyl*-β-*glucosidoxyindole* (*Penta-acetylindican*) (VI).—(*A.*) Potassium 3-β-glucosidoxyindole-2-carboxylate (3·5 g.), fused sodium acetate (3·5 g.), and acetic anhydride (45 c.c.) were heated on the steam-bath for 2 hours, and then at 160—162° for 1 hour. Water (200 c.c.) was added to the cooled mixture; the penta-acetylindican then separated as an oil which gradually solidified. The solid was repeatedly crystallised from methyl alcohol (charcoal) to free it from coloured impurities, and finally from ethyl alcohol, from which it separated in glistening, colourless, elongated, rectangular prisms, m. p. 148° (yield, 4·0 g.) (Found : C, 57·2; H, 5·4. $C_{24}H_{27}O_{11}N$ requires C, 57·0; H, 5·3%). Penta-acetylindican is slightly soluble in cold and easily soluble in hot alcohol, and readily soluble in cold chloroform.

(*B.*) A suspension of methyl 3-tetra-acetyl-β-glucosidoxyindole-2-carboxylate (6 g.) in 5% baryta water (200 c.c.), maintained at 40—45°, was vigorously agitated for 24 hours; the solid gradually dissolved. The excess of barium hydroxide was precipitated by carbon dioxide, the barium carbonate removed, and the filtrate evaporated to dryness. The residue was acetylated and *penta-acetylindican* isolated, m. p. 148°, as described above. Yield, 0·8 g.

(*C.*) A solution of potassium hydroxide (0·84 g.) in water (10 c.c.) was gradually added to one of 1-acetyl-3-hydroxyindole (VI) (*loc. cit.*) (2·1 g.) and tetra-acetyl-α-glucosidyl bromide (6·2 g.) in 100 c.c. of acetone maintained below 5° in an atmosphere of nitrogen. The mixture was kept in the ice-chest for 5 hours and then acidified with dilute acetic acid, and the acetone was removed in a vacuum at room temperature. The dark brown residual oil was dissolved in warm methyl alcohol (20 c.c.), and the solution

poured into cold water (300 c.c.); impure penta-acetylindican was then precipitated in a semi-solid condition. The glucoside crystallised from ethyl alcohol (charcoal) in glistening, colourless, elongated, rectangular prisms, m. p. 148° (yield, 0·7 g.) (Found: C, 56·8; H, 5·3; N, 2·8. $C_{24}H_{27}O_{11}N$ requires C, 57·0; H, 5·3; N, 2·6%). A mixture of the penta-acetylindican prepared in this way with that described above showed no depression of the melting point.

3-β-*Glucosidoxyindole* (*Indican*) (VIII).—1-Acetyl-3-tetra-acetyl-β-glucosidoxyindole (8 g.) quickly dissolved in absolute methyl alcohol (300 c.c.) saturated at room temperature with dry ammonia. After 24 hours, the solution was cooled to 0°, saturated with ammonia, and kept for 12 hours. The ammonia and methyl alcohol were then removed under diminished pressure at 20—25°, and the residual syrup was freed from acetamide by exposure to a high vacuum (1—2 mm.) for 1 hour at 100°. A solution of the green residue in warm water (30 c.c.) was filtered after treatment with charcoal, and exposed to a vacuum over soda-lime and calcium chloride. An almost colourless mass of crystalline indican gradually filled the liquid. Yield, 4·2 g.

The glucoside, after being twice recrystallised from water, was obtained as a mass of colourless, silky needles, m. p. 57—58°. When dried in a vacuum over sulphuric acid for 48 hours, indican lost part of its water of crystallisation; it then melted at 100—101°, became crystalline at 160°, and finally melted at 176—178°. It was obtained anhydrous, m. p. 176—178°, by heating it in the steam-oven for 3 hours, at 110° for 1 hour, and finally at 160° for 5 minutes (Found in a specimen crystallised from water and air-dried: C, 48·2; H, 6·5; N, 4·4; H_2O, 15·8. Calc. for $C_{14}H_{17}O_6N,3H_2O$: C, 48·1; H, 6·6; N, 4·0; H_2O, 15·5%. Found in an anhydrous specimen: C, 56·6; H, 6·0; N, 5·0. Calc. for $C_{14}H_{17}O_6N$: C, 56·9; H, 5·8; N, 4·8%). Anhydrous indican was also obtained from the hydrated variety in colourless prisms, m. p. 176—178°, by crystallisation from warm absolute ethyl alcohol–benzene as described by Perkin and Bloxam (*loc. cit.*).

Synthetic indican is rapidly hydrolysed in warm 3% hydrochloric acid with the liberation of glucose and indoxyl, which in the presence of acid forms "indoxyl brown." If, however, a little ferric chloride is added as an oxygen carrier and air is bubbled through the solution, the liberated indoxyl is instantaneously oxidised to almost pure indigotin. When a drop of concentrated hydrochloric acid is added to a solution of the glucoside in warm glacial acetic acid containing a little *p*-nitrosodimethylaniline as an oxidising agent, glistening leaflets of indigotin, which have a bronze reflex, quickly separate. Hydrolysis of the glucoside in warm 3%

hydrochloric acid in the presence of isatin gives a quantitative yield of indirubin, which separates in microscopic red prisms, whilst, with *p*-nitrobenzaldehyde, the liberated indoxyl forms *p*-nitrobenzaldehydeindogenide, which crystallises from acetone in red, prismatic needles, m. p. 273—274°.

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