PART IV

Steroid glucoside from *Clerodendron infortunatum*. 
Steroid Glucosides from Clerodendron Infortunatum

Clerodendron infortunatum (Fam. Verbenaceae) is a shrub and is commonly known in West Bengal as bhat. The leaves and roots of this plant are used in the indigenous system of medicine for tumors and certain skin diseases. The leaves are also used as vermifuge and as a substitute for chiretta (Swertia chirata). Chemical investigation of the leaves of C. infortunatum in this laboratory led to the isolation of a novel diterpenoid bitter principle called clerodin the structure of which has now been elucidated. Recently Khuda et al. reported the isolation of a sterol, clerosterol, and a number of triterpenoids called clerodol, clerodolone and clerodone from the alcoholic extract of the root of this plant.

In the course of our chemical studies on some plants of the family Verbenaceae, the roots of C. infortunatum were re-examined. From the alcoholic extract of the root of this plant a crystalline sterol glucoside, m.p. 285-90°, has been isolated. It gave violet-green colour in the Liebermann-Burchard test and formed an acetate, m.p. 168-70°, [α]_D = -28° (CHCl_3). Both the glucoside and its acetate showed single spot in TLC experiments on silica gel G using a number of solvent systems. But in TLC over silica gel G impregnated with aqueous silver nitrate solution, the acetate showed two close spots although the glucoside itself showed a single spot. This indicated that the glucoside is a mixture of two closely
related compounds. Acid hydrolysis of the glucoside gave glucose which was identified by paper chromatography and a mixture of two aglycones as shown by two very close spots in TLC, one of which corresponded to that of β-sitosterol.

Recently Suerow reported the isolation of a steroid glucoside, \( C_{35}H_{58}-60O_6 \), m.p. 285-90° (tetra-acetate, \( C_{43}H_{66}-68O_{10} \), m.p. 168-70°, \([\alpha]_D^2 = 27^0\) from the fruits of *Momordica charantia*. The above compound was shown to be a mixture of two glucosides (I & II) which could not be separated. The physical and chemical properties of the glucoside obtained by us from *C. infortunatum* are very close to those obtained from *M. charantia*. For the purpose of direct comparison the steroid glucoside, m.p. 285-90°, was isolated from the fruits of *M. charantia*. The mixed melting points of the glucosides obtained from the two different sources and their corresponding acetates were compared and no depression in melting points were observed. They behaved in the same manner in TLC experiments. The aglycones obtained by acid hydrolysis of the glucosides obtained from the two sources were found to be identical in TLC. Finally the identity of the glucosides were established by comparison of their IR-spectra which were found to be superimposable. Thus the sterol glucosides isolated from *C. infortunatum* were shown to be glucosides of β-sitosterol (I) and \( \Delta^5,25\)-stigmastadien-3β-ol (II). Like Suerow (*loc. cit.*) we also failed to separate the two glucosides.
EXPERIMENTAL

Air-dried powdered roots were extracted in a Soxhlet apparatus with rectified spirit. The extract was concentrated to a small volume and left in cold (5°) overnight when a dark green gummy ppt. was obtained. The supernatant liquid was decanted off and the ppt. was repeatedly extracted with pet. ether (b.p. 60-80°). The above extract was concentrated and was then adsorbed on a column of Brockmann's alumina. The column was eluted with different solvents. Ethanol-chloroform (1:2) eluate gave a material which crystallized from CHCl₃-EtOH mixture, m.p. 285-90°. It gave a single spot in TLC over silica gel G and also on silica gel G impregnated with 12.5 per cent aq. AgNO₃ solution using the solvent systems. (i) benzene:chloroform:methanol (40:10:7 v/v) and (ii) benzene:ethyl acetate:ethanol (50:18:7 v/v). The glucosides isolated from Momordica charantia gave a single spot identical with that of the above glucoside in TLC experiments.

Preparation of acetate. - The acetate was prepared in the usual way by heating the glucoside with pyridine and acetic anhydride and the product was crystallized from chloroform-methanol mixture, m.p. 168-70°, [α]D = 28° (CHCl₃). The acetate gave two spots in TLC over silica gel G impregnated with 12.5 per cent aq. AgNO₃ solution (solvent system used:benzene:chloroform:methanol 50:5:0.5 v/v). The acetate of the glucoside from Momordica
charantia gave two spots identical with those of the above acetate in TLC experiments.

**Acid hydrolysis of the glucoside.** — The acetate (150 mg) was refluxed with ethanolic KOH (5%, 30 ml) and the product was worked up in the usual way when the original glucoside, m.p. 285-90°, was obtained. This glucoside was refluxed with ethanol (18 ml) and conc. HCl (3 ml) for 5 hrs. The alcohol was removed by heating on steam-bath keeping the volume constant with addition of water. The precipitate thus obtained was filtered and crystallized from chloroform-methanol mixture, m.p. 125-29°. It gave two spots in TLC over silica gel G impregnated with 12.5% aq. AgNO₃ solution, identical with those of the aglycone mixture obtained by acid hydrolysis of the glucoside from *Momordica charantia*.

The aqueous filtrate was neutralized with silver carbonate. It was filtered and the filtrate was evaporated to dryness on a steam bath. The residue was extracted with a small amount of dry pyridine. The pyridine solution was used for paper chromatography. The following two solvent systems were used for unidimensional descending paper chromatography.

1. Phenol:water (100:20 v/v)
2. Butanol:water (1246:84 v/v)

Just before use, equal volumes of (a) and (b) were mixed and warmed slightly to form a single phase solvent. Mixture. The chromatogram was sprayed with aniline hydrogen phthalate reagent.
REFERENCES

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