

## THE STEROLS OF JUTE SEED OIL (*CORCHORUS CAPSULARIS*). PART I

BY NIRMAL KUMAR SEN AND JIDAN KUMAR CHAKRAVARTI

A new sterol ( $C_{28}H_{50}O$ , m.p.  $128-29^\circ$ ,  $[\alpha]_D^{20} = -77^\circ.5$ ) has been reported from the non-saponifiable portion of the oil of the seeds of *Corchorus capsularis*.

In course of the study of the component glycerides of jute seed oil (*Corchorus capsularis*) by Sen and Meara (communication to the Journal of Science of Food and Agriculture, 1951) and the component fatty acids of the oil by Chakravarti and Sen (private communication), some quantity of the unsaponifiable matter was isolated from jute seed oil. A few years ago a mixture of sterols (m.p.  $128^\circ$ ) was isolated from the unsaponifiable portion of the jute seed oil by one of the present authors (Sen, this *Journal*, 1928, 5, 759) but owing to the small amount of the material then available no further characterisation of the sterols was possible.

Jute seed oil contains about 3% unsaponifiable matter, which consists of a crystalline substance or substances representing about 20% of the total unsaponifiable and a gummy semi-solid mass which makes up the remainder. The crystalline material consists primarily of a mixture of crude sterols from which two pure sterols melting at  $128-29^\circ$  and at  $116^\circ$  were isolated by chromatographic method. The higher melting sterol was then fractionally crystallised from alcohol and the fractions were worked up as recommended by Anderson *et al.* (*J. Amer. Chem. Soc.*, 1926, 48, 2976, 2987; *J. Biol. Chem.*, 1927, 71, 339) until no further alteration in specific rotation took place. Its molecular formula conforms to  $C_{28}H_{50}O$ , which appears to be isomeric with  $\beta$ - and  $\gamma$ -sitosterols (Sandqvist *et al.*, *Ber.*, 1931, 64, 2167), cinchol (Liebermann, *Ber.*, 1884, 17, 868), cupreol and quebrachol (Windaus, *Ber.*, 1933, 66, 1254, 1689) and rhamnol (Dirscherl, *Z. physiol. Chem.*, 1935, 235, 1), but possesses a higher specific rotation in chloroform  $[\alpha]_D^{20} = -77^\circ.5$  and lower melting point than any one of the isomeric sterols. It gives Liebermann-Burchard reaction and produces with trichloroacetic acid a pale pink colour; with chloral hydrate it displays a change of colour from reddish to pale violet, pale blue, and finally deep blue. It contains one double bond. It forms a digitonide which decomposes without melting at  $215^\circ-20^\circ$ ; a benzoyl derivative, m.p.  $137-39^\circ$ ,  $[\alpha]_D^{20} = -52^\circ.6$  (chloroform) and an acetyl compound, m.p.  $120-22^\circ$ ,  $[\alpha]_D^{20} = -70^\circ.2$  (chloroform). It appears to be a new sterol and further work on this material is in progress.

### EXPERIMENTAL

*Preparation of the Unsaponifiable Matter.*—Refined jute seed oil (300 g.) from *Corchorus capsularis* was saponified by refluxing with ethanolic potassium hydroxide for three hours. The unsaponifiable matter was separated from this soap solution by

extraction with ether by the modified method of Kerr-Sorber (G.S. Jamieson, "Vegetable Fats and Oils", 2nd. Ed., p. 390). The residue (3%) obtained after removal of the solvent was digested with methyl alcohol under reflux for half an hour, and kept in a frigidaire overnight when voluminous white flocculent precipitates separated, and they were collected and washed with a little methyl alcohol. The washings were added to the filtrate and the filtrate was concentrated under reduced pressure on a water-bath. This concentrated solution was kept overnight in a frigidaire when a second lot of precipitate was obtained. There was some gummy residue adhering to the flask and this was collected separately. The two crops of sterols were then dissolved in a moderate quantity of petroleum ether (b.p. 40°-60°) and the solution was left in a refrigerator overnight when beautiful white long needles in cluster arrangement appeared. The product was collected by rapid filtration over a Buchner funnel, washed with a little methyl alcohol and dried in vacuum over anhydrous calcium chloride. The substance melted at 116°-123°, and showed positive Liebermann-Burchard reaction and was precipitated by digitonin.

*Purification by Chromatography.*—A concentrated solution of the sterols in petroleum ether (b.p. 40°-60°) was chromatographed through a column (10 cm.  $\times$  1.5 cm.) of Brockmann alumina. The column had the following characteristics:

(a) Under ordinary light.

|                       |     |                                                                                         |
|-----------------------|-----|-----------------------------------------------------------------------------------------|
| Top 0.5 cm. (approx.) | ... | Sand colour - gradually fainting.                                                       |
| Middle                | ... | Three narrow bands of faint greenish yellow colour separated from each other by 0.5 cm. |

(b) Under ultraviolet rays.

|                       |     |                                                                                    |
|-----------------------|-----|------------------------------------------------------------------------------------|
| Top 0.5 cm. (approx.) | ... | Sand coloured fluorescence.                                                        |
| Middle                | ... | Four narrow greenish yellow fluorescent bands separated from each other by 0.5 cm. |
| Other zone            | ... | Blue fluorescence.                                                                 |

The petroleum ether filtrate from the chromatogram yielded a fraction of sterol on crystallisation having m.p. 116°,  $[\alpha]_D^{20} = -57.9$  (chloroform) and Liebermann-Burchard reaction, positive. The mother-liquor from this operation on complete evaporation left a semi-solid residue of light greenish yellow colour. This substance did not respond to the usual colour reactions of sterol and had its melting point below 100°. From preliminary examination this product was found to be a hydrocarbon residue.

The sand-coloured top layer was removed from the column and worked up to give a very minute sterol fraction responding to colour reactions.

The rest of the column was eluted thoroughly with ether and a major fraction of the sterol was obtained from the elute. This fraction had the following characteristics: m.p. 125-27°;  $[\alpha]_D^{20} = -69.5$  (in chloroform); crystalline clusters of needles when precipitated from ether; positive Liebermann-Burchard reaction; precipitated by digitonin. This fraction was subsequently investigated.

*Fractional Crystallisation.*—The white crystalline product (m.p. 125-27°) obtained from chromatographic separation was fractionally crystallised from alcohol and the top fraction was recrystallised successively from 95% ethyl alcohol several times and their melting points and specific rotations were determined each time. The results are recorded below.

TABLE I

| Crystallisations. | Melting point. | Rotation $[\alpha]_D^{20}$<br>(in chloroform). |
|-------------------|----------------|------------------------------------------------|
| I                 | 126-127°       | -69°.8                                         |
| II                | 126-127°       | -72°.6                                         |
| III               | 126.5-127.5°   | -75°.7                                         |
| IV                | 127-128°       | -77°.2                                         |
| V                 | 128-129°       | -77°.5                                         |

Although the melting points are almost the same in each case, it is evident from the variations in optical rotation that the sterol is not absolutely homogeneous after chromatography. The sterol fraction having maximum rotation (Anderson, *loc. cit.*) thus seemed to be 'pure'. The product was of beautiful shining flakes in appearance and quite stable and had its m.p. 128-29°; specific rotation,  $[\alpha]_D^{20} = -77°.5$  (in chloroform). [Found: C, 83.92; H, 12.15; M.W., 430 (camphor, Rast).  $C_{28}H_{48}O$  requires C, 84.05; H, 12.08 per cent. M.W., 414).

*Digitonide of the Sterol.*—The sterol (0.1 g.) was dissolved in hot 95% alcohol and an equal volume of 1% hot solution of digitouin in 90% alcohol was added to it. The mixture was allowed to cool and left overnight. Heavy flocculent precipitate obtained was filtered, washed well with alcohol and then with a little ether, and dried at 110°. The digitonide, thus prepared, decomposed without melting at 215°-220° with evolution of grey-white fumes.

*Regeneration of the Sterol.*—The digitonide of the sterol was treated with pyridine and refluxed for 4 to 5 hours, cooled and then ether added until a slight turbidity appeared; it was left overnight in a frigidaire and next filtered rapidly. The residue consisted of digitouin which was recovered. The filtrate was treated with dilute hydrochloric acid to remove pyridine and hydrochloric acid was washed away by shaking with portions of water. The ether layer contained the sterol. The ether was evaporated and the residue was treated with tepid methyl alcohol. The solution was filtered and the sterol recrystallised from batches of methyl alcohol had its m.p. 128° and specific rotation,  $[\alpha]_D^{20} = -77°.1$  (in chloroform).

*Sterol Benzoate.*—To ensure the maximum possible purity of the sterol it was benzoylated by the method as recommended by Callow (*Biochem. J.*, 1931, 25, 79) and sterol was recovered by hydrolysis.

Sterol (1.0 g.) was dissolved in 15-20 ml. of dry pyridine and treated with redistilled benzoyl chloride dropwise and the flask was shaken and cooled in ice all the while until a permanent pink colour appeared. The reaction mixture was allowed to stand for 3 hours when the crystals of the sterol benzoate were obtained directly from the reaction flask together with pyridine hydrochloride. This precipitate (fraction I)

was filtered and washed with a little pyridine and the filtrate was allowed to cool to  $-4^{\circ}$ , when a second crop (fraction II) of crystals was obtained. Fractions I and II were well washed with water and finally with alcohol. These aqueous washings were added to the filtrate and a third fraction (III) of the precipitate obtained was collected and washed. The results as observed are shown in Table II.

TABLE II

| Fractions. | Yield.  | Melting point. | Rotation, $[\alpha]_D^{20}$<br>(in chloroform). |
|------------|---------|----------------|-------------------------------------------------|
| I          | 0.60 g. | 136-138°       | -52°.1                                          |
| II         | 0.24    | 133-137°       | -50°.8                                          |
| III        | 0.13    | 125-131°       | -41°.5                                          |

These fractions were worked up as recommended by Callow (*loc. cit.*) and recrystallised from pure ethyl acetate until no further alteration in specific rotation was noted. The sterol benzoate, thus obtained, had its m.p.  $137-39^{\circ}$  and  $[\alpha]_D^{21}$ ,  $-52^{\circ}.6$  (in chloroform). (Found. C, 83.10; H, 10.39.  $C_{28}H_{44}O_2$  requires C, 83.41; H, 10.42 per cent).

**Hydrolysis of the Benzoate.**—Pure benzoate (0.5 g.) was heated on a water-bath with 25-30 ml. of 3% ethanolic caustic potash for about 20 minutes. The sterol, separated on cooling and treating the reaction mixture with water, was washed with water and a little dilute alcohol and recrystallised from 95% ethyl alcohol and dried under vacuum over  $P_2O_5$ . The recovered sterol had its m.p.  $127-28^{\circ}$  and specific rotation in chloroform,  $[\alpha]_D^{21} = -77^{\circ}.3$ .

**Sterol Acetate.**—The sterol (0.2 g.) was heated with 2-3 ml. of pure acetic anhydride (distilled over chalk) in a small porcelain basin covered with a watch glass over a small flame. The solution was allowed to boil slowly for 10-15 minutes and then the excess of acetic anhydride was evaporated off on a water-bath. The contents of the basin were next heated with a few ml. of absolute alcohol in order to prevent immediate solidification, and when cold, treated with water; the solid mass obtained was washed with water and dilute alcohol; finally it was recrystallised from absolute alcohol and dried over  $P_2O_5$ , m.p.  $120-22^{\circ}$ ; specific rotation,  $[\alpha]_D^{21} = -70^{\circ}.1$  (in chloroform).

The sterol regenerated from the acetate by mild hydrolysis with 2% aqueous methanolic caustic potash and crystallised from 95% ethyl alcohol and dried in vacuum had m.p.  $127-29^{\circ}$ ;  $[\alpha]_D^{21} = -77^{\circ}.4$ .

**Colour Reactions of the Sterol.**—The sterol gave positive Liebermann and Liebermann-Burchard reactions and a negative Tortelli-Jaffe test. In the Rosenheim reaction (Rosenheim, *Biochem. J.*, 1929, 23, 47) with trichloroacetic acid it showed a slightly pinkish colour and with chloral hydrate it displayed the colour changes from reddish to pale violet to pale blue and finally deep blue.

**Unsaturation.**—For the determination of unsaturation in the sterol molecule the method of Reindel and Niederlander (*Annalen*, 1929, 478, 147) was employed. A specimen of cholesterol (E. Merck) was taken for a comparative study along with the sterol. The experimental data and results are recorded in the following table.

TABLE III

| Substance.. | Wt.       | N/10-Br<br>in CCl <sub>4</sub><br>added. | N/10-Br<br>in excess. | N/10-Br<br>transformed to HBr<br>in blank. | N/10-Br<br>required<br>to saturate<br>double bond. | N/10-Br required<br>for each<br>double bond. | Number of<br>double bonds<br>found. |
|-------------|-----------|------------------------------------------|-----------------------|--------------------------------------------|----------------------------------------------------|----------------------------------------------|-------------------------------------|
| Chloesterol | 0.0062 g. | 24.97 ml                                 | 17.30 ml.             | 1.20 ml.                                   | 6.49 ml.                                           | 4.97 ml.                                     | 1.3                                 |
| Sterol      | 0.0517    | 15.01                                    | 11.02                 | 1.14                                       | 2.85                                               | 2.49                                         | 1.1                                 |

The number of double bond in the sterol therefore seemed to be one. The slightly higher values obtained might be due to the bromine solution partially oxidising the OH group and forming hydrobromic acid.

The authors wish to acknowledge their indebtedness to Sri Promode Ranjan Das Gupta, M.Sc. of this laboratory for his valuable assistance in the chromatographic purification of the sterols which have been reported in this paper.

CHEMICAL LABORATORY,  
PRESIDENCY COLLEGE,  
CALCUTTA

Received June 15, 1951.