Constituents of the Seeds of Corchorus olitorius, *L. Part II.* Isolation of β-Sitosterol and Corchorolic Acid.*

By GABRA SOLIMAN and WAHBA SALEH.

[Reprint Order No. 4907.]

β-Sitosterol occurs in the seeds of *Corchorus olitorius* and *C. capsularis*. The sterol isolated from the Indian jute seeds and claimed to be new was not found in the Egyptian varieties. The yellow substance obtained from the alcoholic extract of the seeds proved to be a mixture of a phenol and an aliphatic hydroxy-acid, corchorolic acid, $C_{26}H_{52}O_3$, which gave on oxidation a dicarboxylic acid, $C_{26}H_{50}O_4$.

IN conformity with our previous publication * regarding the identity of corchorin with strophanthidin, Chaudhury and Dutta (*J. Indian Chem. Soc.*, 1951, **28**, 167) reported the isolation of corchorin from the Indian jute seeds by a simplified process. Furthermore, they have prepared the acetate of this genin and found that it has the melting point and optical rotation recorded by us. This reveals no difference between the Egyptian and the Indian varieties of *Corchorus*.

Sen (*ibid.*, 1928, **5**, 759) found the oil of the seeds of *Corchorus capsularis* to contain a phytosterol, m. p. 128°. Sen and Chakravarti recently (*ibid.*, 1951, **28**, 727) purified this sterol and characterised it as a new sterol, $C_{29}H_{50}O$, having an acetate, m. p. 122°, a benzoate, m. p. 139°, and a digitonide, m. p. 215—220° (decomp.).

In our search for other constituents in the seeds of *C. olitorius*, we hydrolysed its oil and isolated a phytosterol: the composition of the oil of this variety and the presence of a mixture of sterols had earlier been reported by Sen and Chakravarti (*ibid.*, p. 390). Our sterol was readily identified as β-sitosterol, m. p. 139°, and was characterised as the acetate, m. p. 128°, benzoate, m. p. 147°, and 3 : 5-dinitrobenzoate, m. p. 203°, the identities being established by comparison with authentic specimens (Wallis and Chakravorti, *J. Org. Chem.*, 1937, **2**, 335). Similarly, we have isolated β-sitosterol from the oil of *C. capsularis* of an Egyptian origin (*J.*, 1950, 2199, footnote).

Contrary to expectation, we have failed to isolate Sen and Chakravarti's sterol alongside our β-sitosterol. The absence of this new sterol from the two Egyptian varieties of *Corchorus*, and the absence of β-sitosterol from the Indian jute seeds cannot be reconciled if only due to climatic factors.

In our previous publication (*loc. cit.*) we described a yellow phenol. Attempts to purify this substance by repeated crystallisations led to a gradual fading of its colour and disappearance of its phenolic reaction. However, when its solution in ethyl acetate was shaken with aqueous sodium hydrogen carbonate, a water-insoluble sodium salt of an acid separated. The free acid, m. p. 96°, gave a negative test with ferric chloride and behaved as if it were a saturated aliphatic compound. In another procedure, the crystalline sodium salt was obtained by the action of hot sodium hydroxide solution on the yellow substance. In the same way this acid, now named corchorolic acid, was also isolated from the seeds of *C. capsularis*. Analysis, titration, and acetylation indicate a formula $C_{26}H_{52}O_3$ including a free carboxyl group and a primary or secondary hydroxyl group. Esters were obtained by the action of diazomethane, or of methanol or ethanol and sulphuric acid; analyses of the esters and their acetyl derivatives agree with the proposed formula, but the next lower and higher homologues are not entirely excluded.

The ease of esterification of corchorolic acid and the ready hydrolysis of its esters indicate that the carboxyl group is not attached to a tertiary atom; the stability of methyl corchorolate towards heat in a high vacuum is further evidence that the hydroxyl group is not tertiary. On the other hand, oxidation of corchorolic acid, *via* the methyl ester, with chromium trioxide to a dicarboxylic acid, $C_{26}H_{50}O_4$, m. p. 115°, shows that the hydroxyl

* Part I, *J.*, 1950, 2198.

group is primary. Moreover, the optical inactivity of corchorolic acid and its derivatives might lead to the assumption that its two functional groups are attached to the ends of a normal paraffin chain. This, however, would necessitate the identity of its oxidation product with tetracosane-1 : 24-dicarboxylic acid, m. p. 123·5° (corr.) (Fairweather, *Proc. Roy. Soc. Edinb.*, 1926, **21**, 71): we are as yet unable to confirm this, and the melting points differ considerably.

Experimental

Isolation of β-Sitosterol.—The oil (200 g.) obtained by extraction of the seeds of *C. olitorius* with light petroleum (b. p. 60—80°) was hydrolysed by 4 hours' boiling with 20% alcoholic potassium hydroxide (500 ml.). Most of the alcohol was then distilled off and the residue dissolved in water and extracted with ether. The ethereal solution yielded 6 g. of unsaponifiable matter from which 3·5 g. of the crude sterol, m. p. 105°, were obtained after washing with cold methanol. It crystallised from acetone in plates, m. p. 122—125°. On concentration of the methanol washings or the acetone mother-liquor, the same sterol contaminated with waxy impurities was recovered. The wax was removed by digestion with warm light petroleum (b. p. 40—60°). The insoluble residue, m. p. 135°, crystallised from ethanol in plates, m. p. 139°, $[\alpha]_D^{22}$ −27·5° (*c*, 2·77 in $CHCl_3$), identical with β-sitosterol prepared fron cotton-seed oil (Wallis and Chakravorti, *loc. cit.*). It gave an acetate which crystallised from methanol in plates, m. p. and mixed m. p. 128° (Found: C, 81·6; H, 11·3 Calc. for $C_{31}H_{52}O_2$: C, 81·5; H, 11·5%), $[\alpha]_D^{22}$ −37·6° (*c*, 1·95 in $CHCl_3$), a benzoate, plates (from benzene–methanol), m. p. and mixed m. p. 147°, $[\alpha]_D^{22}$ −11·22° (*c*, 2·84 in $CHCl_3$) (Found: 83·2; H, 10·3. Calc. for $C_{36}H_{54}O_2$: C, 83·3; H, 10·5%), and a 3 : 5-dinitrobenzoate (prepared by 3 : 5-dinitrobenzoyl chloride in pyridine), pale yellowish plates (from benzene–methanol), m. p. and mixed m. p. 203°.

On hydrolysis of the esters with alcoholic potassium hydroxide, β-sitosterol, m. p. 139°, was recovered.

β-Sitosterol was also isolated from the seeds of *C. capsularis* when 100 g. of the oil were saponified as described before. The unsaponifiable matter (3 g.) yielded the crude sterol, m. p. 105°, on treatment with cold methanol. The pure sterol crystallised from ethanol in plates, m. p. 139°, $[\alpha]_D^{22}$ −28·3° (*c*, 2·84 in $CHCl_3$). It gave an acetate, m. p. 128°, $[\alpha]_D^{22}$ −33·9° (*c*, 2·34 in $CHCl_3$) (Found: C, 81·3; H, 11·2. Calc. for $C_{31}H_{52}O_2$: C, 81·6; H, 11·3%), a benzoate, m. p. 147°, and a 3 : 5-dinitrobenzoate, m. p. 203°, all identical with authentic specimens. No other sterol besides β-sitosterol could be obtained from the solvents used in purification or crystallisation.

Isolation of Corchorolic Acid.—The yellowish-brown product (300 g.) obtained from the alcoholic extract (Soliman and Saleh, Part I) of the de-fatted meal (5 kg.) of the seeds of *C. olitorius* was refluxed with ethyl acetate, and the extract was separated by decantation. This process was repeated twice and, after cooling, a sticky brown material separated. The mother-liquor yielded on concentration a yellow solid (80 g.) which was redissolved in ethyl acetate. The solution was decanted from the gummy residue and refluxed with charcoal. The filtrate deposited, on cooling, a yellow micro-crystalline solid, m. p. 103—105°, which gave a green colour with ferric chloride and a yellow colour with potassium hydroxide. It is sparingly soluble in ether or benzene, and dissolves in hot methanol, ethanol, or chloroform, and separates on cooling in a gelatinous form.

Corchorolic acid could not be freed entirely from the phenolic component by repeated crystallisations from ethyl acetate or benzene. When a solution of the yellow solid in ethyl acetate was extracted with sodium hydrogen carbonate solution, sodium corchorolate separated as a jelly. The phenolic component was recovered from the solvent as a yellowish-brown solid. After separation and washing, sodium corchorolate was dissolved in hot dilute acetic acid and heated with charcoal. On cooling of the filtrate, *corchorolic acid* separated as a flocculent precipitate. Crystallised from ethyl acetate it had m. p. 96°, gave a negative test with ferric chloride, and did not decolorise bromine solution in acetic acid. Its solution in pyridine showed no rotation [Found: C, 75·6, 75·5; H, 12·8, 12·6; CO_2H (titration), 11·0, 10·9%; *M* (Rast), 403. $C_{26}H_{52}O_3$ requires C, 75·7; H, 12·7; CO_2H, 10·9%; *M*, 412·4].

For the preparation of corchorolic acid in larger quantities, the yellow solid (20 g.) was boiled gently with 10% aqueous sodium hydroxide (80 ml.) for about 20 min., the mixture becoming brown, and sodium corchorolate (7 g.) separated in glistening fine plates. The acid was liberated and purified as before, and its solution in pyridine showed no rotation (Found: C, 75·6; H, 12·6%).

Acetylcorchorolic acid was prepared by the action of acetic anhydride and pyridine, and crystallised from benzene–light petroleum (b. p. 60—80°) in plates, m. p. 82—83° (Found : C, 74·3; H, 11·95; CO_2H, 9·3. $C_{28}H_{54}O_4$ requires C, 73·9; H, 12·0; CO_2H, 9·5%). Its solution in pyridine showed no rotation.

Methyl Corchorolate.—A suspension of the acid (2 g.) in ether was mixed with ethereal diazomethane and after 3 hr. the solution was washed with 2% aqueous sodium hydroxide and dried. The ester recovered from the ethereal solution crystallised from light petroleum (b. p. 40—60°) in small glistening plates, m. p. 83—84° (Found : C, 75·9, 75·55; H, 12·6, 12·8; OMe, 8·5. $C_{27}H_{54}O_3$ requires C, 76·0; H, 12·8; OMe, 7·3%). *Methyl corchorolate* (same m. p.) was also obtained when the acid (2 g.) in methanol (50 ml.) and concentrated sulphuric acid (3 ml.) was refluxed for 3 hr. It was completely hydrolysed by hot 5% alcoholic potassium hydroxide in 2 hr., the acid melting at 96°. Methyl corchorolate was recovered unchanged after 1 hr. in a high vacuum at 120—125°.

Methyl acetylcorchorolate, prepared as usual, crystallised from light petroleum (b. p. 40—60°) in glistening plates, m. p. 74° (Found : C, 74·7; H, 12·1. $C_{29}H_{56}O_4$ requires C, 74·3; H, 12·05%). It showed no rotation in a benzene solution.

Ethyl Corchorolate.—The acid (1 g.) was refluxed in absolute alcohol (30 ml.) and concentrated sulphuric acid (1·5 ml.) for 3 hr. The *ester* crystallised from light petroleum (b. p. 40—60°) in plates, m. p. 78° (Found : C, 76·7, 76·6; H, 12·6, 13·05. $C_{28}H_{56}O_3$ requires C, 76·3; H, 12·8%). It showed no rotation in benzene solution. Its *acetate* crystallised from light petroleum in plates, m. p. 63—64° (Found : C, 74·9; H, 12·2. $C_{30}H_{58}O_4$ requires C, 74·6; H, 12·1%).

Oxidation of Methyl Corchorolate.—A solution of the ester (2 g.) in hot acetic acid (100 ml.) was cooled to 40°, the ester separating in a gelatinous form. To this mixture, chromium trioxide (2 g.) in acetic acid (40 ml.) and water (2 ml.) was gradually added with stirring, at <40°. Stirring was continued for 3 hr. and the mixture was then kept for about 12 hr. at room temperature, poured into water, and extracted with chloroform. Subsequently, the chloroform solution was extracted with 2% aqueous sodium hydroxide; the sodium salt of the acid ester separated at the interface. The salt was dissolved in hot acetic acid and the solution boiled with charcoal. The filtrate deposited, on cooling, the acid ester (0·8 g.) in plates, m. p. 80—85°. The chloroform solution yielded 0·3 g. of the methyl ester which escaped oxidation.

The acid ester was heated with 10% alcoholic potassium hydroxide for 2 hr. After distillation of the alcohol, the dipotassium salt was separated and acidified with acetic acid. The *dicarboxylic acid* crystallised from acetic acid and recrystallised from ethyl acetate in plates, m. p. 115° [Found : C, 73·5; H, 12·1; CO_2H (titration), 21·3. $C_{26}H_{50}O_4$ requires C, 73·2; H, 11·8; $2CO_2H$, 21·1%]. Its *dimethyl ester*, prepared by diazomethane, crystallised from light petroleum (b. p. 40—60°) in plates, m. p. 69° (Found : C, 74·0; H, 12·1; OMe, 14·9. $C_{28}H_{54}O_4$ requires C, 73·9; H, 12·0; 2OMe, 13·7%). On hydrolysis, the dicarboxylic acid, m. p. 115°, was regenerated.

Corchorolic acid was also separated from the seeds of *C. capsularis* when the yellow substance obtained from its alcoholic extract was analogously treated, but the yield was rather poor.

CHEMISTRY DEPARTMENT, FACULTY OF SCIENCE, ALEXANDRIA UNIVERSITY,
MOHARRAM BEY, ALEXANDRIA. [*Received, December 21st*, 1953.]