289. *Constituents of the Petroleum Extract of* Zea Mays *Stigmas.*

By A. ZAKI and G. SOLIMAN.

Investigation of the petroleum extract of *Zea Mays* stigmas has led to the isolation of a saturated hydrocarbon, m. p. 65°, and a phytosterol mixture from which stigmasterol has been separated, and in which ergosterol has been detected by colour reactions. The presence of a water-soluble acid claimed by other workers has not been confirmed.

RADEMAKER and FISCHER (*Amer. J. Pharm.*, 1886, **16**, 369) have reported that the stigmas of *Zea Mays* contain mazenic acid, which they isolated by extraction of a petroleum or ethereal extract of the stigmas with water. The crystalline form of the acid is described, but its melting point and chemical properties are not recorded.

We were unable to confirm the presence of mazenic acid, but systematic investigation of a petroleum extract of the stigmas (2·2—2·5% yield) led to the isolation of a saturated hydrocarbon, a greenish fatty oil, and a phytosterol mixture. The crude hydrocarbon could be precipitated from the petroleum extract by addition of cold alcohol, and when purified it melted at 65°. It was saturated towards bromine and gave analytical figures corresponding within the usual limits of experimental error to a member of the paraffin series between $C_{29}H_{60}$ and $C_{32}H_{66}$. Its molecular weight, determined cryoscopically in naphthalene, was 450·5. Paraffin hydrocarbons containing both odd and even numbers of carbon atoms have previously been obtained from plant sources, *e.g.*, triacontane, $C_{30}H_{62}$ (m. p. 65°), from *Anthemis nobilis* (Power and Browning, J., 1914, **105**, 1840) and hentriacontane, $C_{31}H_{64}$ (m. p. 68°), from both red clover flowers (Power and Salway, J., 1910, **97**, 1004) and *Clematis vitalba* (Tutin and Clewer, J., 1914, **105**, 1835). A paraffin of m. p. 64° * which appears to be similar to the one now isolated has been obtained from the pollen grains of *Zea Mays* by Anderson (*J. Biol. Chem.*, 1923, **55**, 611), who thought it to be *n*-nonacosane, $C_{29}H_{60}$.

Concentration of the alcoholic filtrate gave a dark greenish solution, which deposited white plates on cooling. The crystalline deposit after purification melted at 147—150° and was shown to be an unsaturated sterol, containing, according to the indications afforded by Rosenheim's reaction (*Biochem. J.*, 1929, **23**, 4379), traces of ergosterol. The presence of a free hydroxyl group in the phytosterol was shown by acetylation (acetate, m. p. 132—134°), and both stigmasterol and sitosterol have been isolated from it by the method of Windaus (*Ber.*, 1906, **39**, 437) and their identity confirmed by comparison with authentic specimens derived from the phytosterol mixture of soya bean.

The presence of the two sterols in the stigmas in approximately equal quantities is interesting in view of the facts that Anderson and Moore (*J. Amer. Chem. Soc.*, 1923, **45**, 1944) and Anderson (*ibid.*, 1924, **46**, 1450) were unable to detect stigmasterol in maize oil, and only traces were isolated by Anderson and Schrinner (*ibid.*, 1926, **48**, 2967) when large quantities of the oil were saponified. Also Anderson (*J. Biol. Chem.*, 1922, **50**, 433;

---

* Krafft (*Ber.*, 1907, **40**, 4778) gives the melting points of the normal paraffin hydrocarbons $C_{29}H_{60}$, $C_{30}H_{62}$, $C_{31}H_{64}$, and $C_{32}H_{66}$ as 64·1°, 65·6°, 68·4°, and 70·5°, respectively.

1923, **55**, 611) found that the oil of the pollen grains does not contain stigmasterol or sitosterol, and in their place isolated a different phytosterol, characterised by its optical inactivity and freedom from water of crystallisation.

EXPERIMENTAL.

Extraction.—The dry powder (1 kg.) of the stigmas was extracted with light petroleum (b. p. 60—80°), and the semi-solid residue left after distillation of the solvent made into a thin paste by the gradual addition of alcohol; more alcohol was then added (1 l. for 25 g.), and the solid separated and washed with alcohol until the filtrate became faintly coloured. The crude solid (0·6—0·8%) was crystallised from absolute alcohol, decolourised by heating with 5% alcoholic potash, and crystallised from acetone. The pure substance melted at 65°, and after drying at 80° in a high vacuum its molecular weight was 450·5 (0·367 g. in 5·202 g. of naphthalene gave Δ 1·085°) (Found: C, 85·3; H, 14·6. Calc. for $C_{29}H_{60}$ or $C_{32}H_{66}$: C, 85·3; H, 14·7%). The hydrocarbon was recovered unchanged after heating with acids, alcoholic alkali, or bromine in glacial acetic acid.

The Phytosterol.—After separation of the hydrocarbon, the greenish filtrate was concentrated by distillation under reduced pressure to one quarter of its volume; on cooling, a crystalline solid separated. After removal of adhering oil on a porous plate and of traces of fat and wax by treatment with cold light petroleum (b. p. 30—50°) the solid (0·05%; m. p. 122—126°) was crystallised seven times from alcohol; it then melted at 147—150°. The phytosterol dissolved freely in ether, chloroform, and benzene and was soluble in hot methyl alcohol, ethyl acetate, and alcohol. It was recovered unchanged after heating with 5% alcoholic potash, lost 3·3% of its weight on drying at 100° in a high vacuum over phosphoric oxide, and had $[\alpha]_{\mathrm{D}}^{21^\circ}$ − 43·8° (l = 1, c = 4·37 in chloroform). In Salkowski's reaction it gave a pink coloration, the acid acquiring a greenish fluorescence, and with the Liebermann–Burchard reagent it gave a violet colour changing to green. Rosenheim's trichloroacetic acid reagent gave a blue colour, and fusion with chloral hydrate resulted in a blue mass after cooling.

The acetate, prepared by heating the phytosterol (2 g.) with acetic anhydride (20 c.c.) and fused sodium acetate (2 g.) for 40 minutes, crystallised from alcohol in plates, m. p. 132—134° (Found: $CH_3{\cdot}CO$, 9·9. Calc. for $C_{31}H_{52}O_2$: $CH_3{\cdot}CO$, 10·6%); $[\alpha]_{\mathrm{D}}^{21^\circ}$ − 47·4° (l = 1, c = 3·73 in chloroform).

Tetrabromostigmasteryl Acetate.—A solution of the phytosterol acetate (5 g.) in dry ether (50 c.c.) was gradually treated with a 5% solution of bromine in glacial acetic acid (65 c.c.) and then cooled for 3 hours in the ice-chest. The bromide which had separated (2·5 g.) crystallised from chloroform–absolute alcohol in small prisms, m. p. 211° (decomp.), not depressed by tetrabromostigmasteryl acetate (Found: C, 48·2; H, 6·4; Br, 41·6. Calc. for $C_{31}H_{50}O_2Br_4$: C, 48·1; H, 6·5; Br, 41·3%); $[\alpha]_{\mathrm{D}}^{17^\circ}$ − 39·4° (l = 2, c = 4·16 in chloroform).

Stigmasteryl Acetate.—A solution of the tetrabromide (1·5 g.) in glacial acetic acid (100 c.c.) was boiled with zinc dust (4 g.) for 6 hours. The acetate, isolated after cooling and dilution with water, crystallised from alcohol in plates, m. p. 144—145°, not depressed by an authentic specimen (Found: C, 81·7; H, 11·1. Calc. for $C_{31}H_{50}O_2$: C, 81·9; H, 11·1%); $[\alpha]_{\mathrm{D}}^{24^\circ}$ − 54·2° (l = 1, c = 3·5 in chloroform).

Stigmasterol.—The acetate (1 g.) was deacetylated by heating with 5% alcoholic potash (30 c.c.) for 45 minutes; after cooling and dilution, the sterol was isolated. It crystallised from alcohol in plates, m. p. 171—172°, which gave no depression on admixture with an authentic specimen and lost 3% of its weight on drying at 100° in a high vacuum over phosphoric oxide (Found: C, 84·1; H, 11·6. Calc. for $C_{29}H_{48}O$: C, 84·4; H, 11·7%); $[\alpha]_{\mathrm{D}}^{24^\circ}$ − 49·5° (l = 1, c = 5·05 in chloroform).

Sitosteryl Acetate.—Crude dibromositosteryl acetate (5 g.) was isolated from the bromination mother-liquor by evaporation of the ether and dilution of the acetic acid solution. It crystallised from alcohol in small yellowish plates, m. p. 122°, and was then debrominated by heating with zinc dust and glacial acetic acid. The acetate, obtained by dilution with water, crystallised from alcohol in small plates, m. p. 133°, not depressed by an authentic specimen (Found: C, 81·4; H, 11·4. Calc. for $C_{31}H_{52}O_2$: C, 81·5; H, 11·5%); $[\alpha]_{\mathrm{D}}^{21^\circ}$ − 38·9° (l = 2, c = 2·76 in chloroform).

Sitosterol.—The acetate was deacetylated by heating with alcoholic potash; the product crystallised from alcohol in plates, m. p. 141°, which lost 2·6% in weight on drying at 100° in a high vacuum over phosphoric oxide and gave no depression of m. p. on admixture with authentic sitosterol (Found: C, 82·9; H, 12·0. Calc. for $C_{29}H_{50}O$: C, 84·0; H, 12·2%. Calc. for $C_{29}H_{50}O,\frac{1}{2}H_2O$: C, 82·5; H, 12·1%); $[\alpha]_{\mathrm{D}}^{22^\circ}$ − 36·6° (l = 2, c = 2·75 in chloroform).

Sitosteryl Bromoacetate.—A solution of the sterol (1 g.) in dry chloroform (20 c.c.) was heated on the water-bath with a slight excess of bromoacetyl chloride (about an hour) until hydrogen chloride ceased to be evolved; the solvent and the excess of the reagent were then removed by distillation under reduced pressure. The residue crystallised from absolute alcohol in plates, m. p. 174°, not depressed by an authentic specimen (Found: C, 69·6; H, 9·3; Br, 15·6. Calc. for $C_{31}H_{51}O_2Br$: C, 69·5; H, 9·5; Br, 14·9%).

The specimens of stigmasterol, sitosterol, and their derivatives used for comparison were obtained from a phytosterol mixture of soya beans supplied by Hansa-Mühle of Hamburg. The sterol mixture, which melted at 140—144°, gave a negative test for ergosterol in Rosenheim's reaction, and after one crystallisation from alcohol melted at 150°; its acetate melted at 136°. The acetate was separated into stigmasteryl acetate and sitosteryl acetate by Windaus's method (*loc. cit.*).

The Fatty Oil.—The fat was obtained from the alcoholic mother-liquor after removal of the sterol and was dried over calcium chloride in a vacuum. The oil, which had a peculiar smell recalling that of the stigmas and solidified at 15°, gave the following constants: acid value, 87·9; saponification value, 148·7; iodine value, 96·2; volatile acid value, 2·5; $n_D^{20°}$ 1·479. Saponification of the oil (50 g.) gave rise to an unsaponifiable fraction, from which a crystalline phytosterol (0·8 g.) was obtained identical with that obtained directly from the oil.

The Ethereal Extract.—The exhausted stigmas were extracted with ether and after evaporation of the solvent a greenish sticky residue was obtained. The extract did not give a crystalline solid on treatment with solvents; in accordance with Rademaker and Fischer (*loc. cit.*) it was boiled with water, but the faintly acidic solution did not leave a crystalline residue on evaporation.

THE EGYPTIAN UNIVERSITY, CAIRO.

[*Received, October 9th,* 1940.]