The products were analyzed for the presence of sulfate ion by digesting with 100 ml. of hot distilled water for one hour to dissolve any $In_2(SO_4)_3$ formed. The mixture then was filtered, washed, and the filtrate tested for the presence of sulfate ion by the usual method. Any sulfide remaining in the residues was converted into sulfate by treatment with bromine and nitric acid according to standard procedure.

The results obtained show that pure In_2S_3 may be heated in air up to 280° with only superficial oxidation. In the temperature range 300–460° the sulfide is gradually oxidized to a mixture of sulfate and oxide, the relative amounts depending on the surface exposed, the temperature and the period of heating. There was no evidence of InS formation.

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Isolation of β-Sitosteryl-D-glucoside from the Juice of Florida Valencia Oranges (*Citrus sinensis*, L.)[1]

BY LYLE JAMES SWIFT

Steryl glycosides or phytosterolins have been reported by Matlack in the rinds[2] and in the pulp[3] of California Valencia oranges and by Nolte and von Loesecke[4] in the juice of Florida Valencia oranges. In none of these publications were the glycosides completely identified. Matlack[2] described the sterol from the rind glycoside as sitosterol, but did not identify the sugar component. He surmised that the glycoside was identical with the sitosterol-D-glucoside synthesized by Salway.[5] It is apparent from data in Table I that Matlack's glycoside is very similar, if not identical, with the glucoside described in this paper. Since the various sitosterols were not known when either Salway or Matlack did their work, it seems likely that the former used a mixture of sitosterols in his preparation and the latter, by omitting the specific rotation measurements, failed to detect the discrepancies in rotation between the synthetic glucoside and the β-sitosterol-D-glucoside that he probably had isolated.

The pulp phytosterolin isolated by Matlack he described simply by the necessary classification reactions and a melting point of 250–255°. Nolte and von Loesecke[4] described their phytosterolin also by only the classification reactions.

It was the aim of the present work to establish beyond doubt the identity of the components of the steryl glycoside occurring in orange juice. The glucose was identified by qualitative tests and quantitative determinations after hydrolysis. The identification of the β-sitosterol depends upon the close agreement of its physical properties and those of its derivatives with the values obtained by other investigators.

All melting points determined and reported in this study were taken with a thermometer calibrated by the Bureau of Standards. Correction for stem exposure was also made.

Experimental

Isolation of β-Sitosteryl Glucoside.—About 1100 kg. of pasteurized juice from the 1947 crop of Florida Valencia oranges was filtered in batches through buchner funnels, using a commercial filter-aid. The filter-aid with the retained matter was then extracted with acetone which was subsequently evaporated, leaving the colored lipids of the juice and considerable aqueous solution. Upon extraction of this mixture with petroleum ether (b.p. 35–60°) and subsequent concentration of the extract to recover the lipids, some of the β-sitosteryl glucoside precipitated and was separated, washed with petroleum ether, and recrystallized twice from 90% ethanol. The yield at this point was 26.0 g. of the glucoside from the 1000 g. of lipid matter obtained. A further quantity of the glucoside was isolated from 600 g. of lipid after saponification, extraction of the unsaponifiable matter, and acidification when extraction of the acids was attempted. The glucoside collected at the interface and tended to cause troublesome emulsions. It was separated by filtration, washed with 95% ethanol, and recrystallized from a mixture of ethanol and pyridine. The weight was 8.9 g. of lipid, or 14.8 g. on the basis of the whole 1000 g. Thus, there was obtained a total of 40.8 g. of β-sitosteryl glucoside from 1100 kg. of juice, or a yield of 0.0037%.

The β-sitosteryl glucoside was insoluble in most solvents, was sparingly soluble in 95% ethanol (about 0.06 g. in 100 ml. at 20° and 0.225 g. in 100 ml. in hot), but soluble in pyridine. It gave positive Lieberman–Burchard and Molisch reactions. The melting point was dependent on the rate of heating. In a bath preheated to 298° the glucoside melted in 20 to 30 seconds; $[\alpha]^{20}D$ −40.1° (pyridine, 1.334 g./100 ml., 2-dm. tube).

Anal. Calcd. for $C_{35}H_{60}O_6$: C, 72.85; H, 10.49. Found: C, 72.26, 72.21; H, 10.40, 10.36.

β-Sitosteryl Glucoside Tetraacetate.—A 2.0-g. portion of the glucoside was dissolved in 25 ml. of pyridine and 15 ml. of acetic anhydride was added. The mixture was refluxed for one hour, the solvents were removed by heating under vacuum, and the residue was dissolved in hot 95% ethanol. It was then treated with decolorizing carbon, filtered and allowed to stand. The crystals that formed melted at 170° and the yield was 2.3 g. The m.p. could only be raised to 171° by repeated recrystallization from alcohol. The $[\alpha]^{25}D$ −33.7° (pyridine, 1.3012 g./100 ml. 2-dm. tube) and a Rast determination of the molecular weight using palmitic acid as the solvent gave a value of 787.

Anal. Calcd. for $C_{43}H_{68}O_{10}$: C, 69.30; H, 9.20; CH_3CO, 23.09. Found: C, 69.23, 69.32; H, 9.09, 9.29; CH_3CO, 22.95, 22.86.

The regenerated β-sitosteryl glucoside, which had come out of solution as the saponification proceeded, was separated by filtration, recrystallized from a pyridine–alcohol mixture, and dried. The m.p. was 296° and the $[\alpha]^{27}D$ −41.8° (pyridine, 1.1228 g./100 ml., 2-dm. tube).

TABLE I

COMPARISON OF CONSTANTS OBTAINED IN THE PRESENT WORK WITH THOSE OF SALWAY[5] AND MATLACK[2] ON CORRESPONDING COMPOUNDS

	Present work		Matlack[2]		Salway[5]	
	(α)	M.p., °C.	(α)	M.p., °C.	(α)	M.p., °C.
Sitosterol glucoside	−40.1°	298	..	280	....	295–300
Sitosterol glucoside tetraacetate	−33.7	171	..	164–165	−22.9	166–167
Sitosterol glucoside tetrabenzoate	+15.9	201	..	198	+18.3	198
Sitosterol	−38.2	137–138	..	136–137	−32.2	138
Sitosterol acetate	−40.3	125–126	..	124–125	....	.....
Sitosterol benzoate	−13.6	147–148	..	.....	....	.....

(1) Report of a study made under the Research and Marketing Act of 1946.

(2) M. B. Matlack, *J. Am. Pharm. Assoc.*, **18**, 24 (1929).

(3) M. B. Matlack, *J. Org. Chem.*, **5**, 504 (1940).

(4) A. J. Nolte and H. W. von Loesecke, *Food Research*, **5**, 457 (1940).

(5) A. H. Salway, *J. Chem. Soc.*, **103**, 1022 (1913).

β-Sitosteryl Glucoside Tetrabenzoate.—A 3.0-g. portion of the β-sitosteryl glucoside was dissolved in 35 ml. of pyridine and 25 ml. of benzoyl chloride was added. The mixture was heated at 100° for about 30 minutes with occasional stirring and then poured into water. When the precipitate had settled, the aqueous solution was decanted and the residue was dissolved in ether. The ethereal solution was washed with dilute sodium carbonate and then with water. After evaporation of the ether, the residue was twice recrystallized from ethanol. The yield was 4.2 g. The m.p. was 201°, $[\alpha]^{25}D$ 15.9° (chloroform, 1.3095 g./100 ml., 2-dm.).

Anal. Calcd. for $C_{63}H_{76}O_{10}$: C, 76.21; H, 7.71; C_6H_5CO, 42.33. Found: C, 75.93, 75.77; H, 7.68, 7.69; C_6H_5CO, 41.45, 41.63.

Hydrolysis of the β-Sitosteryl Glucoside.—A 2.1692-g. sample of the β-sitosteryl glucoside was hydrolyzed by the method of Thornton, *et al.*[6] The glucoside was refluxed for 22 hours with 100 ml. of absolute ethanol and 1 ml. of concentrated sulfuric acid, solution gradually taking place as the reaction progressed. The bulk of the ethanol was distilled off under reduced pressure and the residue was diluted with water and extracted with several portions of ether. The ethereal solution was washed with water and the washings added to the main solution which was then further acidified with 1 ml. of concentrated sulfuric acid and refluxed for five hours to hydrolyze any ethyl glucoside. The aqueous solution was cooled and made to 200 ml. volume. This solution was used in establishing the identity of the sugar.

Identification of Glucose from Glucoside.—A Shaffer–Hartmann titration of an aliquot representing 0.2169 g. of the original glucoside showed it to contain 0.0661 g. of reducing sugar, calculated as glucose, or 97.6% of the theoretical amount. A specific rotation based on this concentration gave a value of +52.4°, accepted value, +52.2°.

A portion of the hydrolysate was oxidized to saccharic acid and was converted to the characteristic boat-like crystals of its potassium acid salt as directed in Morrow and Sandstrom.[7]

When adjusted to 0.2 molar concentration, the hydrolysate was indistinguishable from a known glucose solution in time of osazone formation and microscopic appearance of the osazone.

β-Sitosterol Component of the Glucoside.—The washed ether extract of the hydrolysis mixture from 2.1692 g. of the glucoside was evaporated and dried in a tared flask and found to weigh 1.503 g. (98%). This crude sterol was found to be 84.4% precipitable with digitonin (conversion factor, 0.257). Another sample of the crude sterol was recrystallized from ethanol and chromatographed over asbestos paper, developing with 1% ethanol in petroleum ether. Streaking with concentrated sulfuric acid showed only one component. Another sample of the crude sterols, acetylated and then brominated in an ether–glacial acetic acid mixture, showed no insoluble tetrabromides after 48 hours at 0–5°, hence the presence of a doubly-unsaturated sterol is unlikely.

For purification and preparation of derivatives, 61.0 g. of crude sterol glucoside was hydrolyzed as before and the sterols recrystallized from ethanol, petroleum ether and acetone until all that could be crystallized melted between 135 and 138° and weighed 35.1 g. (80%). Repeated recrystallizations from acetone failed to raised the m.p. above 137–138°; $[\alpha]^{20}D$ −38.2° (chloroform, 5.0693 g./100 ml., 2-dm. tube).

Anal. Calcd. for $C_{29}H_{50}O$: C, 83.98; H, 12.16. Found: C, 84.28, 84.23; H, 12.00, 12.11.

β-Sitosterol Acetate.—This was prepared in the usual way, m.p. 125–126°, $[\alpha]^{22}D$ −40.3° (chloroform, 1.6824 g./100 ml., 2-dm. tube).

Anal. Calcd. for $C_{31}H_{52}O_2$: C, 81.52; H, 11.48. Found: C, 81.53, 81.69; H, 11.33, 11.34.

Saponification of the acetate regenerated the β-sitosterol unchanged.

β-Sitosterol Benzoate.—This compound was prepared by heating β-sitosterol with benzoyl chloride and pyridine. After purification the benzoate melted at 147–148°. The $[\alpha]^{21}D$ was −13.6° (chloroform, 2.0760 g./100 ml., 2-dm. tube).

Anal. Calcd. for $C_{36}H_{54}O_2$: C, 83.34; H, 10.49. Found: C, 83.36, 83.31; H, 10.49, 10.43.

Saponification of the benzoate regenerated the β-sitosterol unchanged.

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(6) M. H. Thornton, H. R. Kraybill and J. H. Mitchell, THIS JOURNAL, **62**, 2006 (1940).

(7) C. A. Morrow and W. M. Sandstrom, "Biochemical Laboratory Methods," John Wiley and Sons, Inc., New York, N. Y., 1935, pp. 165–166.

The Standard Potentials of the Silver–Silver Chloride Electrode in Absolute Volts

BY D. F. SWINEHART

The standard potentials of the cell $Pt-H_2(g)/HCl(m)/AgCl-Ag$ have been accurately determined by Harned and Ehlers.[1] These constants are important for the determination of the thermodynamic properties of hydrochloric acid solutions and because they are used in the accurate determination of the ionization constants of weak acids and bases.

In 1948 the National Bureau of Standards officially changed from the international to the absolute system of electrical units.[2] All standard cells are now certified in absolute volts. It seems desirable to recalculate the results of Harned and Ehlers in the new units. The function 2.30259 RT/F in international volts used by these investigators was evaluated from the constants listed by Lewis and Randall.[3,4] These values, however, due to a partial cancellation of errors, accidentally agree within one part in 10^5 with the presently accepted values of the function in absolute volts.

Therefore, their results have been recalculated in international volts using the values of 2.30259 RT/F given in those units by Manov, Bates, Hamer and Acree.[5] This was done by calculating for a few points at the highest dilution values for the function which Harned and Ehlers used in extrapolating to $m = 0$ to obtain $E°$. This was done in the units used by them and again in international volts. The consistent small differences were then added to Harned and Ehlers' values for $E°$ to obtain $E°$ in international volts. This, in effect, uses their entire extrapolation procedure. These results are shown in the third column of Table I.

These results were fitted by least squares by the equation used by Harned and Ehlers yielding the result: $E°$ (international volts) $= 0.22247 - 6.4450 \times 10^{-4}(t - 25) - 3.276 \times 10^{-6}(t - 25)^2 + 8.99 \times 10^{-9}(t - 25)^3$. Values of $E°$ calculated

(1) H. S. Harned and R. W. Ehlers, THIS JOURNAL, **55**, 2179 (1933).

(2) National Bureau of Standards Circular No. 475, 1949.

(3) G. N. Lewis and M. Randall, "Thermodynamics," McGraw-Hill Book Co., Inc., New York, N. Y., 1923.

(4) H. S. Harned and D. D. Wright, THIS JOURNAL, **55**, 4849 (1933).

(5) G. G. Manov, R. G. Bates, W. J. Hamer and S. F. Acree, *ibid.*, **65**, 1765 (1943).