Isolation of β-Sitosterol from Chufa (*Cyperus esculentus* L.) Tubers

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The chemical constitution of the tubers of *Cyperus esculentus* L., also known as chufa, tiger nut, edible cyperus, rushnut, and earth almond, has received sporadic attention through many years. The principal component is the oil, sometimes called sedge oil, which forms about 25% of the tubers and which was valued as a food and for lubrication purposes.[1] The detailed analysis of the oil and other constituents of the tubers cultivated in Egypt will be shortly reported elsewhere.[2]

An early publication by Baughman and Jamieson[3] dealing with the fatty acid constituents of the oil mentioned a phytosterol that was isolated by saponification of the unsaturated acid fraction. These authors reported for the crystalline product m.p. 134–135° and for its acetyl derivative m.p. 122–123°. As it appears that the product has not been examined by later workers, we wish now to report on its identity.

Saponification of the oil (about 25% of the tubers, by light petroleum extraction) with alcoholic potassium hydroxide gave a nonsaponifiable fraction as a bright yellow oil in 0.68% yield. By chromatographic fractionation of this material, there has been obtained β-sitosterol, m.p. 136–137°, $[\alpha]_D^{28}$ −34°. It afforded an acetate, m.p. 126–127°, $[\alpha]_D^{28}$ −41°, and a benzoate, m.p. 142–4°, $[\alpha]_D^{28}$ −15°. The properties of the compound, its acetate, and benzoate agree with those reported by Bernstein and Wallis[4] for β-sitosterol isolated from cottonseed oil; moreover, the melting points of the alcohol and the acetate were undepressed by the authentic samples. The infrared absorption spectrum[5] (Nujol) of the alcohol contained bands at 3400 cm.$^{-1}$ (OH), 840 and 803 (trisubstituted olefin), and was identical with that of authentic material. It is likely, therefore, that β-sitosterol is the material which has been isolated by Baughman and Jamieson.[3]

(1) J. Pieraerts, *L'Agronomie Coloniale*, **9**, No. 67, 7 (1923); H. Winter, *Z. Lebensmittel-Unters. u.-Forsch.*, **105**, 200 (1957); F. R. Earle *et al.*, *J. Am. Oil Chemists' Soc.*, **36**, 304 (1959).

(2) A preliminary report, A. M. Gad and F. Osman, *Egypt. J. Chem.*, **2**, No. 1, 123 (1959), will be followed by more details.

(3) W. F. Baughman and G. S. Jamieson, *J. Agric. Research*, **26**, 77 (1923).

(4) S. Bernstein and E. S. Wallis, *J. Org. Chem.*, **2**, 341 (1937–1938).

(5) We thank Professor F. G. Baddar, Ein-Shams University, for the infrared measurements.

EXPERIMENTAL[6]

β-Sitosterol. Exhaustive extraction of the finely ground tubers of *Cyperus esculentus* L. with light petroleum (b.p. 70–80°) removed about 25% as oil. Saponification of the oil with ethanolic potassium hydroxide followed by work-up in the usual manner gave 0.68% of a bright yellow thick oil. Percolation of a benzene solution of 0.683 g. of the nonsaponifiable matter through an alumina column and prolonged washing with the same solvent removed 0.368 g. of wax and oily unsaturated hydrocarbon material. Final stripping of the column with 3% methanol in benzene removed 0.268 g. of a colorless solid. Recrystallization from methanol gave β-sitosterol as colorless plates, m.p. and mixed m.p. 136–137°, $[\alpha]_D^{28}$ −34° ($CHCl_3$); ϵ_{max} = 3,200(204 mμ); in ethanol; (reported[4] m.p. 136–137°, $[\alpha]_D^{28}$ −36°). The infrared absorption spectrum (Nujol) was identical with that of an authentic sample.

β-Sitosteryl acetate was prepared by treating a pyridine solution of β-sitosterol with acetic anhydride at 100° for 1 hr. The product, isolated by the usual work-up, was crystallized from methanol to give colorless needles, m.p. and mixed m.p. 126–127°, $[\alpha]_D^{28}$ −41° ($CHCl_3$), ϵ_{max} = 3400 (204 mμ, ethanol); (reported[4] m.p. 125–126°).

β-Sitosteryl benzoate was prepared by heating a mixture of β-sitosterol, pyridine, and benzoyl chloride on the water bath for 1 hr. followed by isolation in the usual manner. Crystallization of the product from methanol gave needles, m.p. 142–144°, $[\alpha]_D^{28}$ −15° ($CHCl_3$); (reported[4] m.p. 145°).

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(6) Melting points are uncorrected. Infrared spectra were determined on a Perkin-Elmer Infracord 137 spectrophotometer.

The Orientation of the Isopropyl Group of Dihydroabietic γ-Lactone

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Treatment of commercial, partially hydrogenated rosin or dihydroabietic acids with strong mineral acid has led to dihydroabietic α-lactone (the lactone of "hydroxytetrahydroabietic acid"[1c]).[2–7] The con-

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(2) (a) L. Ruzicka and J. Meyer, *Helv. Chim. Acta*, **5**, 315 (1922); (b) L. Ruzicka, H. Waldmann, P. J. Meier, and H. Hosli, *Helv. Chim. Acta*, **16**, 169 (1933).

(3) E. E. Fleck and S. Palkin, *J. Am. Chem. Soc.*, **61**, 1230, 3197 (1939).

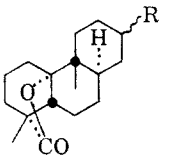
(4) R. Lombard and J. Ebelin, *Bull. soc. chim. France*, 930 (1953), and references contained therein.

(5) L. Velluz, G. Muller, A. Petit, and J. Mathieu, *Bull. soc. chim. France*, 401 (1954).

(6) Le-van-Thoi, *Bull. soc. chim. France*, 439 (1954).

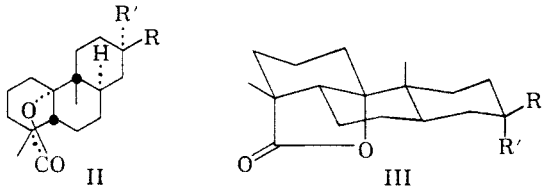
(7) R. F. B. Cox (assigned to Hercules Powder Co.), U. S. Patent 2,355,782.

figuration of all asymmetric centers of the lactone, except that to which the isopropyl side chain is attached, has been assigned (*cf.* Ia).[8] Recently oxidation experiments have been reported which affected exclusively this side chain of undetermined configuration.[9] In two of the three reaction products the isopropyl function had been converted into a hydroxyisopropyl group (cf. Ib) and into an acetyl group (cf. Ic), respectively. The structural relationship of these two oxidation products has been established by the conversion of the latter (Ic) into the former (Ib) with methyl magnesium iodide and hydrolysis.[9]



Ia. R = $CH(CH_3)_2$
b. R = $C(OH)(CH_3)_2$
c. R = $COCH_3$

In view of the small likelihood of the oxidation of the isopropyl group having affected its neighboring asymmetric carbon atom, the above data indicate the stereochemistry of Ia, b, and c to be identical. Hence the elucidation of the configuration of the side chain of any of these substances would determine the stereochemistry for all. This has now been accomplished. Equilibration of the ketone Ic in refluxing methanolic sodium methoxide solution left the starting material unchanged. This fact implies that the acetyl group possesses the equatorial β configuration. Furthermore, formula IIa, IIIa in conformational form, represents the stereostructure of dihydroabietic γ-lactone.

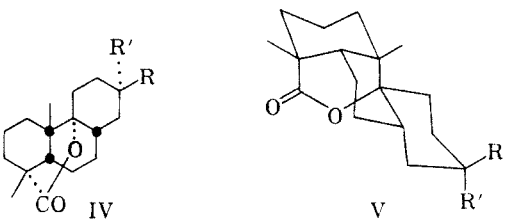


II III

a. R = $CH(CH_3)_2$, R′ = H
b. R = $C(OH)(CH_3)_2$, R′ = H
c. R = C_2H_5, R′ = CH_3
d. R = CH_3, R′ = C_2H_5

Strong acid treatments of pimaric and isopimaric acids have been shown to lead to a hydroxy γ-lactone among other products.[10,11] Comparison of its infrared spectrum, melting point, and mixed melting point with those of the hydroxylactone, obtained by the oxidation of dihydroabietic γ-lactone,[9] showed these substances to be identical. Hence, structure IIb, IIIb in conformational representation, can be assigned now to the hydration product of the pimaric acids.

It has been reported[12] that treatment of dihydroabietic γ-lactone with concentrated sulfuric acid yielded a δ-lactone. Its structure can be represented now by IVa, conformationally by Va. Were the conversion of the γ- into the δ-lactone assumed to be high-yielding, a configuration of the isopropyl group opposite to that depicted in IIa–Va might be deduced, since, among other changes, a bulky isopropyl group is inverted from an equatorial into an axial conformation.[13] However, a preliminary study of the acid-catalyzed equilibration of the lactones reveals an equilibrium mixture containing only 25–50% δ-lactone. Furthermore, the following simple theoretical calculation leads to a similar conclusion.



IV V

a. R = $CH(CH_3)_2$, R′ = H
b. R = C_2H_5, R′ = CH_3
c. R = CH_3, R′ = C_2H_5

A recent study of the equilibrium position between the five- and six-membered lactones derived from dihydropimaric and dihydroisopimaric acids has afforded these data[11]: IIIc⇌Vb: 95.0 ± 0.6% δ-lactone; IIId⇌Vc: 96.4 ± 0.8% δ-lactone. The average between these values, 95.7 ± 0.8% δ-lactone, corresponds to the equilibrium position of the lactones of a hypothetical system II–V, where R = R′, e.g. R = R′ = H. In this system the six-membered lactone would be favored by 1.8 ± 0.1 kcal. at equilibrium. Since the introduction of an axial isopropyl group into such a δ-lactone implies its de-stabilization by two more skew nonbonded interactions, *i.e.*, *ca.* 1.8 kcal.,[14] the resulting compound should have the same energy content as its γ-lactone counterpart. Hence, the equilibrium mixture of the dihydroabietic lactones should contain *ca.* 50% δ-lactone.

EXPERIMENTAL

Equilibration of ketolactone Ic. A solution of 7.6 mg. of ketolactone Ic, m.p. 135–136.8°, $[\alpha]_D$ −15.4° (chloroform), and sodium methoxide, from 0.76 mg. of sodium, in 0.5 ml. of methanol was refluxed under nitrogen for 1.5 hr. The mixture then was neutralized with 1% methanolic hydrogen chloride, the solvent evaporated, and the white residue ex-

(8) L. A. Subluskey and T. F. Sanderson, *J. Am. Chem. Soc.*, **76**, 3512 (1954).

(9) J. Minn, T. F. Sanderson, and L. A. Subluskey, *J. Am. Chem. Soc.*, **78**, 630 (1956).

(10) E. E. Fleck and S. Palkin, *J. Am. Chem. Soc.*, **62**, 2044 (1940).

(11) E. Wenkert and J. W. Chamberlin, *J. Am. Chem. Soc.*, **81**, 688 (1959).

(12) Le-van-Thoi, *Bull. soc. chim.*, 761 (1955).

(13) Indeed, this type of assumption has led to an erroneous assignment of the configuration of the isopropyl group in the dihydroabietic acid precursor of IIa.[11]

(14) C. W. Beckett, K. S. Pitzer, and R. Spitzer, *J. Am. Chem. Soc.*, **69**, 2488 (1947).

tracted with ether. Removal of the solvent gave 6.6 mg. of colorless crystalline material. Crystallization of the latter from aqueous acetone and aqueous methanol yielded 4.4 mg. of colorless crystals, m.p. 136–137.2°, $[\alpha]_D$ −19.2° (chloroform), no depression of mixed melting point with starting material, infrared spectrum (chloroform) identical with that of starting ketone.

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Acenaphthene Arsenicals

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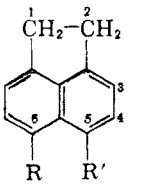
Several years ago, we gave some attention to the preparation of a number of acenaphthene arsenicals.[1] More recently,[2] we have repeated and extended this work. A search of *Chemical Abstracts* reveals no mention of acenaphthene arsenic compounds, a somewhat surprising situation, considering the number of acenaphthene compounds now in existence. Part of our interest in the series lies in the fact that the dimethylene bridge of acenaphthene can be oxidized into various forms some of which ought to prove of value as insecticidal or antifungal agents.

Since direct aromatic substitution of acenaphthenes produces, largely, 5-substituted compounds and disubstitution the 5,6- compounds, all of the compounds prepared by us belong to one of these structure types. The chemical literature describes satisfactorily the preparation of 5-nitroacenaphthene.[3] This compound was found to be reduced nicely in the laboratory to the corresponding amine either by low pressure hydrogenation using Raney nickel or palladium-charcoal, or by refluxing in hydrazine with the same catalysts. Other methods of reduction were found to be less satisfactory.

The 5-aminoacenaphthene was then converted into acenaphthene-5-arsonic acid, the first of the new compounds (I), *via* the Bart reaction on the diazotized amine. Application of the Scheller reaction (acetone solvent, arsenic trichloride, cuprous bromide catalyst) gave very poor yields even though conditions were varied extensively. The method of arsonation proceeding through the diazonium fluoborate (decomposition in aqueous as well as nonaqueous media) failed to give appreciable yields.

Acenaphthene-5-arsonic acid was then converted to the 5-dichloroarsine (II) and the 5-dibromoarsine (III) using the appropriate phosphorus trihalide



I. R = H, R′ = AsO_3H_2
II. R = H, R′ = $AsCl_2$
III. R = H, R′ = $AsBr_2$
IV. R = H, R′ = AsI_2
V. R = NO_2, R′ = AsO_3H_2

in organic media. Attempts to convert the arsonic acid into the diiodoarsine (IV) with hydriodic acid in glacial acetic acid gave a tan product melting at 95–96°, but having an analysis some five or more per cent high in iodine. There is some question of the method to be employed in the analysis for iodine in the presence of arsenic, but the compound was probably contaminated with elemental iodine or arsenic iodide.

The direct nitration of acenaphthene-5-arsonic acid was then attempted, leading to the supposed 6 - nitroacenaphthene - 5 - arsonic acid (V). After several exploratory runs, the correct conditions were established and a product giving the proper nitrogen content was obtained.

Several attempts to oxidize acenaphthene-5-arsonic acid into a naphthalic acid derivative with chromic anhydride failed to yield isolable material.

Structure proof for the supposed 6-nitro-5-arsonic acid based on the known 6-nitro-5-amine[4] is planned for the near future.

EXPERIMENTAL

Melting points were obtained on a Fisher-Johns melting point apparatus calibrated against pure compounds of known melting points.

Acenaphthene. Technical grade material was purchased from the Reilly Tar and Chemical Corporation of Indianapolis, and was recrystallized from glacial acetic acid with a charcoal treatment; m.p. 92–93°.

5-Nitroacenaphthene. This was prepared in 66% yield by direct nitration of acenaphthene using essentially the method of Sachs and Mosebach,[3] m.p. 101–102°.

5-Aminoacenaphthene. I. *Palladium-hydrazine method.* Five grams (0.033 mole) of 5-nitroacenaphthene was dissolved in 60 ml. absolute ethanol and 5 ml. of 95% hydrazine was added. The solution was heated to near reflux temperature and 0.05 g. of 10% palladium on charcoal (Matheson Coleman & Bell) was added. The mixture was refluxed for 10 min., an additional 0.05-g. portion of catalyst was added and reflux was continued for 90 min. The material was then treated with charcoal, filtered hot, and poured slowly into 800 ml. of cold water. The white solid separating was filtered, washed with water, and dried to give a product (3.6 g., 84% yield) melting at 104–105°.

II. *Palladium or raney nickel with low-pressure hydrogen.* Five grams (0.033 mole) of acenaphthene was placed with 60 ml. of absolute alcohol in the pressure bottle (400 ml. capacity) of a low-pressure Parr hydrogenation apparatus and 0.1 g. of 10% palladium-on-charcoal or approximately 0.5 g. of Raney nickel[5] was added. Hydrogen gas at 35 p.s.i.g. was supplied and the suspension was shaked for 90 min. The

(1) G. W. Batzis, Master's Thesis, Xavier University, Cincinnati, Ohio, 1952.
(2) J. O. Kroeger, Master's Thesis, Xavier University, Cincinnati, Ohio, 1959.
(3) F. Sachs and G. Mosebach, *Ber.*, **43**, 2473 (1910).
(4) H. J. Richter, *J. Org. Chem.*, **21**, 619 (1956).
(5) R. Mozingo, *Org. Syntheses*, **21**, 15, (1941).