The Conversion of 1-Phenyl-2-isopropylcyclopropane to 1,1,2-Trimethylindan.—While stirring, 14.4 g. of 1-phenyl-2-isopropylcyclopropane was run into 30 ml. of 90% sulfuric acid. Within a half hour the temperature rose to 40° after which it was maintained at 35° for one hour. The residual oil was removed by adding 25 ml. of ligroin, separating from the acid, washing the ligroin layer twice with 5-ml. portions of cold, concentrated sulfuric acid, steam distilling, separating the volatile oil, removing the ligroin by distillation through a short Widmer column, and distilling the residue. This gave 3.2 g. of 1,1,2-trimethylindan, b. p. 208°, d^{20}_4 0.919, $n^{20}\mathrm{D}$ 1.5137.

Analyses.[11] Calculated for $C_{12}H_{16}$: C, 89.9; H, 10.1. Found (1-phenyl-2-isopropylcyclopropane): C, 89.7; H, 10.1. Found (1,1,2-trimethylindan): C, 89.7; H, 10.2.

Oxidation of 0.46 g. of the product obtained above by the method of Price, Davidson and Bogert[4] yielded 0.30 g. of the ketone acid (V) which when recrystallized from water melted at 156°. This did not depress the melting point of an authentic sample of the acid melting at 158°. Both samples gave iodoform when treated with sodium hypoiodite.

Summary

1. 1-Phenyl-2-isopropylcyclopropane is rearranged by 90% sulfuric acid to 1,1,2-trimethylindan.

2. Under similar conditions 1-phenyl-2-methylcyclopropane and 1-phenyl-2-ethylcyclopropane undergo polymerization.

3. Cyclopropanes obey the modernized Markovnikov rule.

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[11] These analyses were performed by Mr. Saul Gottlieb, Columbia University.

[CONTRIBUTION FROM THE KEDZIE CHEMICAL LABORATORY, MICHIGAN STATE COLLEGE OF AGRICULTURE AND APPLIED SCIENCE]

The Isolation of Stigmasterol and β-Sitosterol from the Common Bean, *Phaseolus vulgaris*

BY ARNOLD C. OTT[1] AND CHARLES D. BALL

The content of the "crude fat" of the common or navy bean, *Phaseolus vulgaris*, has been reported by various workers[2] to be between 1.4 and 2.36%. Grimme[3] submitted values for certain physical and chemical constants of the oil among which was an unsaponifiable matter percentage of 5.85. Likiernik[4] appeared to be the only worker to have isolated sterols from any part of the bean. He obtained from the seed coats two substances named by him paraphytosterol and phasol. In harmony with the work of Anderson and Shriner[5] paraphytosterol would appear to be γ-sitosterol, while phasol, m. p. 189–190°, $[\alpha]\mathrm{D}$ + 30.6°, is either a mixture or has not been identified with any of the better known sterols. Attention has been focused on the ether soluble portion of the navy bean by the recent observation of Bowman,[6] who maintains that the bean oil retards the digestion of soluble starch by pancreatic amylase *in vitro*.

In the present work the bean oil, "ether extract," has been found to represent about 2.6% of the bean, of which 5.0 to 6.0% was unsaponifiable matter. This unsaponifiable matter consisted of 55 to 60% crude sterols. Stigmasterol made up about one-fourth of the sterols, while β-sitosterol was found to be present to the extent of 5 to 7% of the total sterols. The separation of the stigmasterol was based on the insolubility of the tetrabromide while the β-sitosterol was obtained from the mother liquors after debromination and fractional crystallization as acetates. No sterols corresponding to α-sitosterols appeared to be present in any appreciable amount. After irradiation the unsaponifiable matter had a vitamin-D activity of about 700 U. S. P. units per g. of unsaponifiable matter, thus indicating the presence in very small amount or amounts of irradiatable sterol or sterols.

Experimental

1. **Extraction of the Beans.**—Forty kg. of the common bean, *Phaseolus vulgaris*, was ground in a hammer mill and extracted six times by cold ethyl ether. The extract was filtered and the solvent distilled off in an atmosphere of carbon dioxide. The yield was 0.83 kg. of yellow oil, which represented 1.9% of the original beans.

2. **Saponification.**—To the entire lot of the oil 4 liters of ethanol and 1 liter of 50% potassium hydroxide were added. The mixture was stirred on the steam-bath for two hours. Water was then added and the unsaponifiable matter was exhaustively extracted from the soaps with "Skellysolve B." The combined extracts were concentrated to 50% concentration of oil and then washed free from alkali. The remainder of the solvent was evaporated; yield, 48.7 g. of a yellow, semi-solid oil. This quantity represented about 5.9% of the crude oil.

3. **Preparation of Crude Sterols.**—The entire lot of unsaponifiable material was taken up in petroleum ether, b. p. 40–60°, and steam passed into the solution until saturation nearly occurred. The solution was then allowed to stand overnight. The first fraction of crystals, designated Fraction I, consisted of 22.5 g. of colorless crystals; m. p. 138–144°; $[\alpha]^{27}\mathrm{D}$ −44.0° (35.4 mg., 2.1 ml. chloroform, l = 1 dm., $\alpha^{27}\mathrm{D}$ −0.742°, *average* reading). The Liebermann–Burchard reaction was strongly positive. The mother liquor was evaporated to 300 ml. and allowed to stand for twenty-four hours in the cold. The result was Fraction II, which consisted of 7.2 g. of crystals, m. p. 128–131°; $[\alpha]^{26}\mathrm{D}$ −38.0° (38.6 mg., 2.1 ml. chloroform, l = 1 dm., $\alpha^{26}\mathrm{D}$ −0.698°, *average* reading). A third fraction of about 2 g. was obtained from the mother liquors of

[1] Present address: Hercules Powder Co., Wilmington, Del.

[2] (a) Atwater and Bryant, *U. S. Dept. Agr. Off. Exp. Sta. Bul.*, 28 Rev. (1906); (b) Peterson and Churchill, THIS JOURNAL, **43**, 1180 (1921); (c) Eichelberger, *ibid.*, **44**, 1407 (1922).

[3] Grimme, *Pharm. Zentr.*, **52**, 1141 (1911).

[4] (a) Likiernik, *Ber.*, **24**, 187 (1891); (b) Likiernik, *Z. physiol. Chem.*, **15**, 426 (1891).

[5] Anderson and Shriner, THIS JOURNAL, **48**, 2976 (1926).

[6] Bowman, *Science*, **98**, 308 (1943).

fraction II. The total crude sterols amounted to 29–30 g. or about 60% of the unsaponifiable matter.

4. **Separation of Sterols.**—An unsuccessful attempt was made to separate the sterols as azobenzenemonocarboxylic esters according to the chromatographic method of Ladenburg, Fernholz and Wallis.[7] From their work the separability of sterols on alumina depends primarily upon differences between the number of double bonds, and not upon their position or upon the nature of the side chains. Inference might be implied that the sterols of the common bean are all of the same general type.

One and one-half g. portions from each of Fractions I and II were combined, and acetylated with 60 ml. of acetic anhydride, by refluxing for one and one-half hours. The mixture was concentrated to saturation and after cooling for one hour at 12°, the crude acetates were filtered off. The acetates were brominated by solution in 30 ml. of ethyl ether and the addition of 38 ml. of bromine–acetic acid solution (5 g. bromine in 100 ml. of glacial acetic acid) following the procedure of Windaus and Hauth.[8] After refrigeration the insoluble bromides were filtered and washed with cold ethyl ether. A yield of 1.4 g., m. p. 190–194°, was obtained. Six recrystallizations of the bromides from chloroform–methanol gave a product with a m. p. of 194–196°.

Stigmasteryl Acetate.—To a solution of 1.2 g. of the recrystallized bromides in 12 ml. of glacial acetic acid 1.2 g. of zinc dust was added. The mixture was refluxed for one and one-half hours, filtered hot, diluted with water, and extracted with ethyl ether. The ether layer was washed with dilute sodium sulfite, then with water, and the ether removed by evaporation. The product obtained weighed 580 mg. It was recrystallized four times from ethanol, and twice from 2–1 methanol–chloroform; m. p. 139–140°; $[\alpha]^{25}_{\mathrm{D}}$ −54.0° (37.4 mg., 2.1 ml. chloforoform, $l = 1$ dm., α^{25}_{D} −0.962°, *average* reading).

Anal.[9] Calcd. for $C_{31}H_{50}O_2$: C, 81.88; H, 11.09. Found: C, 81.18; H, 10.73.

Stigmasterol.—Four hundred mg. of the above acetate was hydrolyzed for one hour with 10% alcoholic potassium hydroxide. Water was added and the mixture extracted with ethyl ether. The ether solution was washed with dilute sodium carbonate, and then with water. After removal of the ether, the residue was recrystallized three times from 95% ethanol, m. p. 168–169°; $[\alpha]^{29}_{\mathrm{D}}$ −47.3° (28.6 mg., 2.1 ml. chlofororm, $l = 1$ dm., α^{29}_{D} −0.644°, *average* reading).

Anal. Calcd. for $C_{29}H_{48}O$: C, 84.40; H, 11.72. Found: C, 83.47; H, 11.70.

Stigmasteryl Benzoate.—About 300 mg. of the free sterol was dissolved in 2 ml. of dry pyridine; then 0.6 ml. of benzoyl chloride was added. The mixture was heated for two and one-half hours in a glass-stoppered flask on the steam-bath and allowed to cool. The contents were poured into ice-cold 5% sulfuric acid. The solution was extracted with ethyl ether, washed with dilute sodium carbonate, and then with 10% ethanol. The extract was evaporated on the steam-bath and the residue recrystallized three times from ethanol and once from methanol–chloroform; m. p. 160.5–161.5°; $[\alpha]^{24}_{\mathrm{D}}$ −24.5° (152.3 mg., 2.1 ml. chloroform, $l = 1$ dm., α^{24}_{D} −1.779°, *average* reading).

Anal. Calcd. for $C_{36}H_{52}O_2$: C, 83.65; H, 10.14. Found: C, 83.95; H, 9.83.

β-Sitosteryl Acetate.—The remainders of Fractions I and II were combined with the small amount of the third fraction. This combined fraction was converted into acetates by treatment with 250 ml. of acetic anhydride and refluxing for two and one-half hours. On cooling, a yellow, wax-like cake appeared on the surface, while suspended below colorless crystals were observed. The colorless crystals were dissolved by warming the solution to about 40°, and the colored, wax-like cake removed by filtration. The filtrate was reduced in volume to effect a more complete crystallization, cooled in the refrigerator for one hour, filtered and washed with cold glacial acetic acid. The product obtained (about 10 g.) was brominated as previously described, the brominated products filtered off, and the derivatives in the filtrate debrominated in the usual manner with zinc dust. The debrominated acetates were taken up in sufficient 1-1 acetone–chloroform to effect a solution about one-half saturated. After standing overnight, the solid acetates were removed by filtration. The acetates were subjected to a triangular fractionation[10] first with a 3–1 acetone–methanol mixture and subsequently with a 3–1 ethanol–benzene mixture. From this fractionation appeared: (1) a wax-like material, insoluble in cold concentrated sulfuric acid, with a m. p. of 65–66.5° after thirteen recrystallizations from ethanol–benzene and (2) β-sitosteryl acetate; m. p. 123–124°; $[\alpha]^{28}_{\mathrm{D}}$ −38.5° (40.5 mg., 2.1 ml. chloroform, $l = 1$ dm., α^{28}_{D} −0.744°, *average* reading).

Anal. Calcd. for $C_{31}H_{52}O_2$: C, 81.52; H, 11.47. Found: C, 81.59; H, 10.96.

The first material was no further characterized.

β-Sitosterol.—The above acetate was saponified with 10% alcoholic potassium hydroxide, extracted and washed as usual. The product was crystallized three times from methanol–chloroform; m. p. 136–138°; $[\alpha]^{25}_{\mathrm{D}}$ −37.8° (19.2 mg., 2.1 ml. chloroform, $l = 1$ dm., α^{25}_{D} −0.346°, *average* reading).

Anal. Calcd. for $C_{29}H_{50}O$: C, 83.99; H, 12.15. Found: C, 83.41; H, 11.25.

β-Sitosteryl Benzoate.—Fifty mg. of the free sterol was treated with 0.2 ml. of benzoyl chloride in 1.5 ml. of dry pyridine. The mixture was heated for one and one-half hours on the steam-bath and allowed to stand. The contents of the reaction were poured into cold 5% sulfuric acid and the mixture extracted with ethyl ether. The residue obtained from the ether extraction was crystallized four times from ethanol; m. p. 145–146°; $[\alpha]^{25}_{\mathrm{D}}$ −13.5° (19.4 mg., 2.1 ml. chloroform, $l = 1$ dm., α^{25}_{D} −0.125°, *average* reading).

In Table I are presented the data on the properties of stigmasterol and β-sitosterol and their derivatives as obtained or derived from the common bean, together with a comparison of the corresponding products as obtained from other sources by other investigators.

TABLE I

	M. p., °C.	$[\alpha]_{\mathrm{D}}$ in $CHCl_3$
Stigmasterol (isolated from the common bean)	168–169	−47.3
acetate	139–140	−54.0
benzoate	160.5–161.5	−24.5
Stigmasterol[11]	169–170	−44.7 to 51.0
acetate[11]	141 to 144	−55.6
benzoate[11]	160	
β-Sitosterol (isolated from the common bean)	136–138	−37.8
acetate	123–124	−38.5
benzoate	145–146	−13.5
β-Sitosterol[11]	139–140	−36.1
acetate[11]	127–128	−39.1[12]
Unknown sterol (isolated from the common bean)	155–157	−55.8
acetate	134–135	
dinitrobenzoate	222–223	−17.3

Attempted Isolation of α-Sitosterols.—From the crystallization by triangulation as described under the isolation of

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[7] Ladenburg, Fernholz and Wallis, *J. Org. Chem.*, **3**, 294 (1938).

[8] Windaus and Hauth, *Ber.*, **39**, 4378 (1906).

[9] Microanalyses are by Dr. T. S. Ma, University of Chicago

[10] Morton, "Laboratory Techniques in Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1938, p. 162.

[11] Sobotka, "The Chemistry of the Sterids," The Williams and Wilkins Co., Baltimore, Md., 1938, pp. 224 and 239.

[12] Anderson, THIS JOURNAL, **48**, 2987 (1926).

β-sitosteryl acetate, the fraction containing the soluble acetates was obtained by evaporation of the solvent on the steam-bath. The residue obtained was taken up in ethanol and hydrolyzed by the addition of alcoholic potassium hydroxide in the manner previously described. The solution obtained was worked up in the same manner as given for β-sitosterol. The product was dried and treated with sufficient 3,5-dinitrobenzoyl chloride in order to yield the corresponding esters. Triangular fractionation was again used in an attempt to isolate one of the three known "α-sitosterols," which according to Wallis and Fernholz[13] differ greatly in the solubilities of their 3,5-dinitrobenzoates. The most insoluble of the 3,5-dinitrobenzoates obtained had a m. p. of 222–223°; $[\alpha]^{28}\text{D}$ −17.3° (23.6 mg., 2.1 ml. chloroform, $l = 1$ dm., $\alpha^{28}\text{D}$ −0.195°, *average* reading). The regenerated free sterol melted at 155–157°; $[\alpha]^{25}\text{D}$ −55.8° (15.6 mg., 2.1 ml. chloroform, $l = 1$ dm., $\alpha^{25}\text{D}$ −0.415°, *average* reading). The acetate of the free sterol was prepared; m. p. 134–135°. The Liebermann–Burchard reaction was positive. Since all the derivatives of all the "α-sitosterols" have a positive value of relatively high magnitude for their optical rotations, the absence of any appreciable amounts of α-sitosterols is indicated, and the presence of an unknown sterol is indicated. A summary of the properties of this unknown sterol is also included in Table I.

5. Irradiation of the Unsaponifiable Matter.—A portion of the unsaponifiable matter was dissolved in ethyl ether and irradiated with ultraviolet light. Aliquots of this stock solution were taken for assay by the standard biological method. The results of the assay indicated an activity of 700 U. S. P. units per g. of crude unsaponifiable matter.[14]

Summary

1. The total unsaponifiable matter of the common bean, *Phaseolus vulgaris*, is about 5.9%, or about 0.15% of the air-dry bean.
2. About 60% of the unsaponifiable matter is represented by crude sterols.
3. Stigmasterol and β-sitosterol have been isolated and characterized, the former representing about 25%, and the latter about 6% of the crude sterols.
4. An unidentified sterol with a high negative optical rotation has been isolated.
5. Neither α-sitosterols nor γ-sitosterol, the apparent paraphytosterol of Likiernik, have been found, although the presence of small amounts of these sterols is not precluded.
6. The unsaponifiable matter of the common bean after irradiation has a Vitamin-D activity of 700 U. S. P. units per g.

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[13] Wallis and Fernholz, THIS JOURNAL, **58**, 2446 (1936).

[14] The authors wish to thank Dr. C. A. Hoppert, Professor of Biological Chemistry, for the Vitamin-D assay.

EAST LANSING, MICH. RECEIVED JANUARY 3, 1944

[CONTRIBUTION FROM THE DEPARTMENTS OF CHEMISTRY OF THE UNIVERSITY OF TEXAS AND SOUTHWESTERN LOUISIANA INSTITUTE]

Studies in the Biphenyl Series. IV. The Iodination of the Acetate, Benzoate and Benzenesulfonate of 4-Hydroxybiphenyl[1]

BY HAROLD R. SCHMIDT, CORA MAY SAVOY[2] AND JOHN LEO ABERNETHY

In connection with an investigation of the bactericidal activity of certain halo-4-hydroxybiphenyls, it was of interest to find a more convenient route to 4-hydroxy-4′-iodobiphenyl than had been used previously. Since it is known that chlorination[3,4,5] and bromination[6,7] of certain esters of 4-hydroxybiphenyl give rise to substitution in the 4′-position of the biphenyl nucleus, and subsequent hydrolysis gives the desired 4′-chloro and 4′-bromo compound, it seemed that iodination of these esters might give rise to the corresponding iodo compounds. Hence, 4-acetyloxybiphenyl, 4-benzoyloxybiphenyl and 4-benzenesulfonyloxybiphenyl were subjected to iodination.

Iodination of 4-acetyloxybiphenyl (I) with iodine in the presence of nitric acid gave rise to 4-acetyloxy-4′-iodobiphenyl (II) when either carbon tetrachloride or glacial acetic acid was used as the solvent. Hydrolysis of the ester gave rise to 4-hydroxy-4′-iodobiphenyl (III) which did not depress the melting point of the known compound prepared from benzidine (IV), by converting IV to 4-amino-4′-iodobiphenyl (V) and then to III, according to the method of van Alphen.[8] Furthermore, acetylation of III produced an ester which did not depress the melting point of the product of iodination of I. It was found that a better yield of the iodinated ester (II) was obtained if nitric acid was added immediately to the reaction mixture rather than dropwise over a period of several hours. The latter procedure gave rise to a considerable amount of nitrated product, along with the iodinated ester.

By means of a similar reaction, 4-benzoyloxybiphenyl (I) was converted to 4-benzoyloxy-4′-iodobiphenyl (II). Benzoylation of III gave rise to this same ester. Hydrolysis of II yielded III. Similarly, iodination of 4-benzenesulfonyloxybiphenyl (I) yielded 4-benzenesulfonyloxy-4′-iodobiphenyl (II), which was proved by benzenesulfonation of III. Hydrolysis of II yielded III.

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[1] A portion of this paper was abstracted from a thesis presented by Harold R. Schmidt to the Graduate Faculty of the University of Texas in partial fulfillment of the requirements for the degree of Master of Arts, August, 1943, and was presented before the Texas Academy of Science, November, 1943, in Austin, Texas.

[2] Present address: Department of Chemistry, Southwestern Louisiana Institute, Lafayette, Louisiana.

[3] Savoy and Abernethy, THIS JOURNAL, **64**, 2219 (1942).

[4] Savoy and Abernethy, *ibid.*, **64**, 2719 (1942).

[5] Schmidt, Savoy and Abernethy, *ibid.*, **65**, 296 (1943).

[6] Hazlet, *ibid.*, **59**, 1087 (1937).

[7] Hazlet, Alliger and Tiede, *ibid.*, **61**, 1447 (1939).

[8] van Alphen, *Rec. trav. chim.*, **50**, 1111 (1931).