original aqueous solution and this solution was evaporated to dryness on the steam-bath. The residue was exhaustively extracted with absolute alcohol, and the residual sodium chloride was discarded. Upon evaporation of the alcohol there was obtained an oil, which was soluble in dry acetone. This oil became crystalline on long standing in a desiccator, after which treatment with dry acetone yielded two fractions. The acetone insoluble fraction upon analysis proved to be the hydrochloride of 5-methyl-2-hydroxy-thiazoline. The acetone soluble fraction, which melted at 39°, proved to be its acid insoluble keto form. It was recrystallized from dry acetone and benzene.

TABLE I

Compound	M. p., °C. (uncor.)	Formula	N Analyses, % Calcd.	Found
5-Methyl-2-phenylmercapto-thiazoline				
Hydrochloride	171	$C_{10}H_{12}NS_2Cl$	5.69	5.57
Picrate	141	$C_{16}H_{14}O_7N_4S_2$	12.55	12.65
5-Methyl-2-(2′-methyl)-phenylmercapto-thiazoline				
Hydrochloride	164	$C_{11}H_{14}NS_2Cl$	5.39	5.75
Picrate	133	$C_{17}H_{16}O_7N_4S_2$	12.38	12.46
5-Methyl-2-(3′-methyl)-phenylmercapto-thiazoline				
Hydrochloride	139	$C_{11}H_{14}NS_2Cl$	5.39	5.32
Picrate	118	$C_{17}H_{16}O_7N_4S_2$	12.38	12.48
Sulfonic Acid		$C_{11}H_{13}O_3NS_3$	4.61	4.38
5-Methyl-thiazolidone-2				
Keto form[a]	39	C_4H_7ONS	11.96	12.12
Enol, hydrochloride	204	C_4H_8ONSCl	9.12	9.35

[a] Calcd.: C, 40.98; H, 6.02; S, 27.37. Found: C, 41.13; H, 6.08; S, 27.79.

Acknowledgment.—The authors desire to thank Merck and Company, Inc., Rahway, New Jersey, for a research fellowship.

Summary

Thiophenols on condensation with allyl mustard oil have been found to yield phenylmercapto-thiazolines, in contrast with phenols, which yield thiazolinephenols. These heterocyclic thio-ethers do not rearrange in the presence of the usual acidic rearranging agents, but are readily hydrolyzed in alkaline solution.

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The Sterols of Alfalfa Seed Oil. II. Isolation of β-Spinasterol and δ-Spinasterol

BY L. CARROLL KING AND CHARLES D. BALL

In a previous communication[1] from this Laboratory the isolation of α-spinasterol from the unsaponifiable fraction of alfalfa seed oil was reported. In addition to α-spinasterol we have now obtained two other isomeric sterols, β-spinasterol[2] and a third substance whose properties indicate that it has not before been isolated. Since this new sterol is closely related to the known α- and β-spinasterols in structure and properties we have designated it as δ-spinasterol.

In order to separate these three isomeric substances, the crude alfalfa seed oil sterols were dissolved in a large excess of acetic anhydride. On cooling this mixture, the acetates of α-spinasterol and β-spinasterol separated as flaky crystals and were filtered off while the δ-spinasteryl acetate remained for the most part in the acetic anhydride mother liquors. α-Spinasterol and β-spinasterol were separated by taking advantage of the greater solubility of the latter in 85% ethanol.

Each of the three isomeric spinasterols was isolated from two different sources—first, from Hardigan alfalfa seed oil and second, from Grim alfalfa seed oil. The crude sterols from Hardigan alfalfa seed oil consisted of about 23% α-spinasterol, 39% β-spinasterol and 6% δ-spinasterol. The amounts of the corresponding substances isolated from the crude sterols of Grim alfalfa seed oil were 17, 28 and 4.5%, respectively.

The chemical reactions and relationships of α-, β- and δ-spinasterol are summarized in Fig. 1.

δ-Spinasterol was purified by fractional crystallization from 95% ethanol and from methanol. The purest product obtained had a melting point of 142–143° and $[\alpha]^{19}D$ 6.15°. It precipitated with digitonin and gave a positive Liebermann–Burchard test.

The analytical data for δ-spinasterol and its derivatives indicated the formula $C_{29}H_{47}OH \cdot {}^1/_2H_2O$ for the sterol. On catalytic reduction of the acetate in acetic acid solution, a compound identical with α-stigmastenyl acetate[3] (I) was obtained. δ-Spinasterol is, therefore, a doubly

(1) King and Ball, THIS JOURNAL, **61**, 2910 (1939).
(2) Heyl and Larsen, *J. Pharm. Assoc.*, **22**, 510 (1933).
(3) Fernholz and Ruigh, THIS JOURNAL, **62**, 2341 (1940).

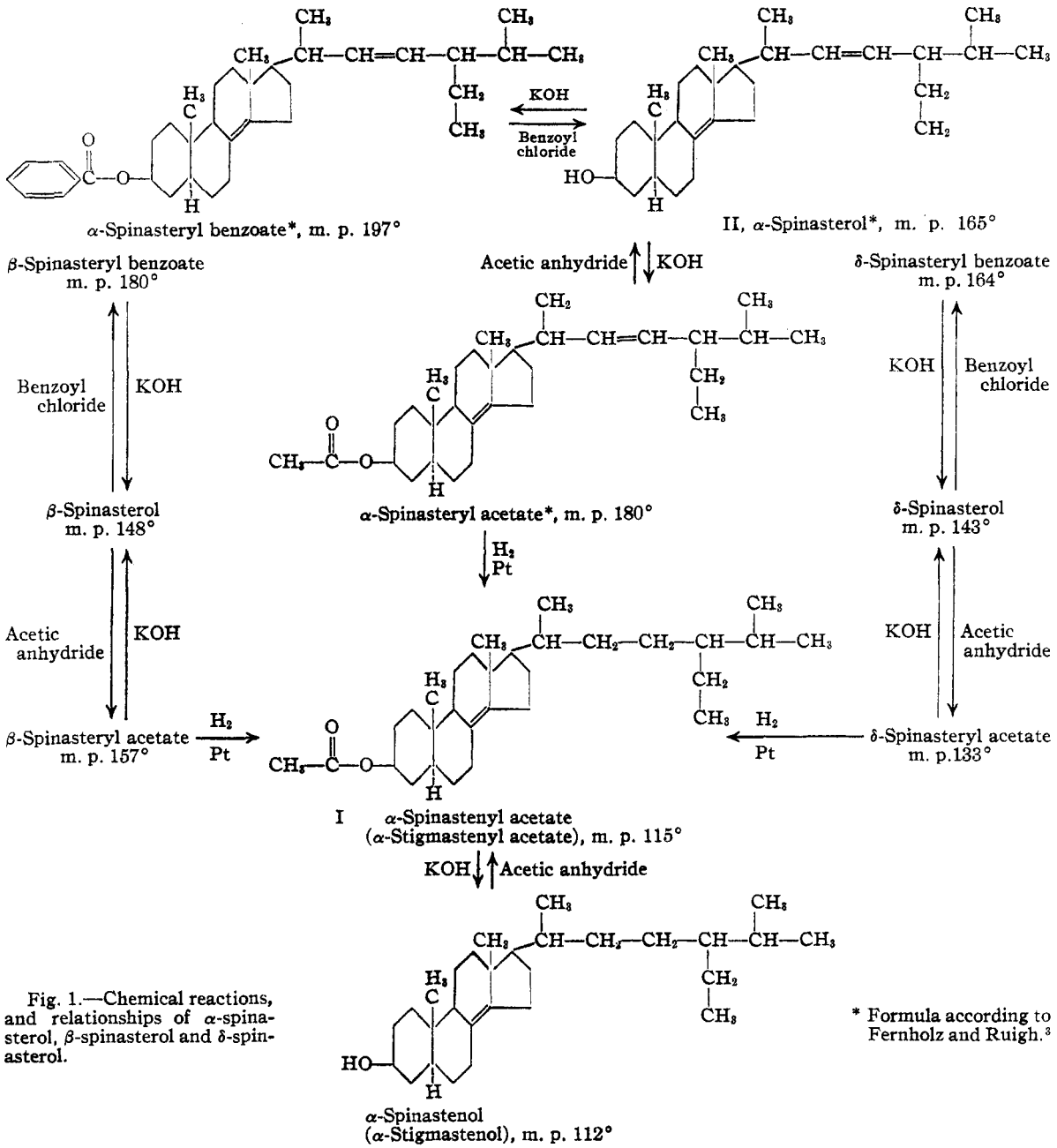


Fig. 1.—Chemical reactions, and relationships of α-spinasterol, β-spinasterol and δ-spinasterol.

* Formula according to Fernholz and Ruigh.[3]

unsaturated sterol having the same structural configuration as α-spinasterol except for the position of the double bonds. It has one double bond located at position Δ8:14 or in a position from which it can shift easily into the Δ8:14 configuration, when in the presence of hydrogenating catalysts.

The identity of the β-spinasterol isolated from alfalfa seed oil with that isolated from spinach fat is indicated by correspondence in physical properties, Table I, and also by similar behavior when subjected to catalytic reduction.

TABLE I

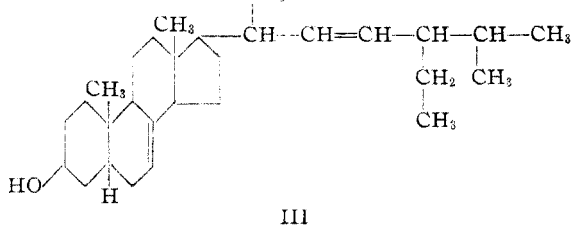
	M. p., °C.	$[\alpha]_D$ in $CHCl_3$, °C.	$[\alpha]_{5461}$, °C.
β-Spinasterol (isolated from alfalfa seed oil)	148–150	5.9	...
Acetate	153–155	5.1	...
Benzoate	181–183	7.5	...
β-Spinasterol (isolated from spinach fat)	145–148	...	7.65
Acetate	150–154	...	7.2

The β-spinasterol isolated from spinach fat gave analytical data for the formula $C_{29}H_{47}OH$·

$^1/_2H_2O$ or $C_{29}H_{47}OH$. On catalytic reduction of the acetate in acetic acid solution α-stigmastenyl acetate (I) was obtained. β-Spinasterol is, therefore, a doubly unsaturated sterol, related in structural configuration to α-spinasterol except for the position of the double bonds.

Recently Kuwada and Yosiki[4] reported that the bessisterol, isolated by them[5,6] from the rhizomes of *Mormordica cochinchenses* Spreng, was identical with α-spinasterol. The correspondence in chemical and physical properties of bessisterol and α-spinasterol is very good except that the specific rotation of bessisterol is $[\alpha]_D$ $-13.5°$, and that of bessisteryl acetate is $[\alpha]_D$ $-13.47°$, whereas the specific rotation of α-spinasterol is usually reported at $[\alpha]_D$ $+1.7°$ to $-3.5°$ and α-spinasteryl acetate at $[\alpha]_D$ $-4.7°$. In this Laboratory α-spinasterol was repeatedly recrystallized from methanol and from chloroform–methanol in an attempt to bring its rotation to a value similar to that reported for bessisterol. This attempt was unsuccessful; the best value obtained was $[\alpha]_D$ $-2.7°$. An attempt to obtain α-spinasteryl acetate with a specific rotation similar to the value reported for bessisteryl acetate also failed.

Fernholz and Ruigh[3] proposed for α-spinasterol the structures (II) and (III). It is of



III

interest to note that the specific rotation of structure II, calculated according to the method of Bernstein, Kauzmann and Wallis,[7] is $[\alpha]_D$ $8.2°$ while that of structure III is $[\alpha]_D$ -10.9. Neither of these values can be correlated with the observed value for α-spinasterol, but structure III is in fair agreement with the observed value for bessisterol.

Experimental

1. Separation of Crude Sterols from the Unsaponifiable Fraction.—One hundred and fifty grams of crude unsaponifiable material from Hardigan alfalfa seed oil, prepared as directed by King and Ball,[1] was dissolved in enough ethyl ether to make about 500 cc. A stream of water vapor[8] was passed through the solution until a slight turbidity occurred. After the mixture had stood overnight at 5°, the crystalline mass which separated was filtered off and recrystallized from ethyl ether. The original mother liquors and those from the subsequent recrystallizations were concentrated somewhat and the whole process repeated. In this way 26 fractions were obtained.

The 26 solid fractions weighing about 47 g. were nearly free of colored oily material. The first fraction isolated melted about 158°; subsequent fractions had lower and lower melting points, while the last fractions had very indefinite melting ranges in the vicinity of 60°. These crude solid fractions were combined and classified into two groups, those melting above 120° (Fraction A) and those melting below 120° (Fraction B). Fraction B appeared to consist of hydrocarbons, sterols and other alcohols. Further work on it is in progress in this Laboratory.

2. Fractionation of the Crude Steryl Acetates from Cold Acetic Anhydride.—The crude sterol fractions melting above 120° (Fraction A) were combined and dissolved in acetic anhydride (30 cc. per gram). This mixture was heated one hour, allowed to stand overnight, and then filtered. The solid steryl acetates (Fraction A_1) so obtained consisted mostly of α-spinasteryl and β-spinasteryl acetates; yield 24.5 g.; m. p. 152–157°.

The acetic anhydride mother liquors from the above were hydrolyzed by heating with water. The solid steryl acetates precipitated by this treatment (Fraction A_2) were filtered off and dried; yield 2.9 g.; m. p. 122–127°.

3. Separation and Identification of α-Spinasterol and β-Spinasterol. α-Spinasterol.—The crude acetates, m. p. 152–157° (Fraction A_1), were hydrolyzed by boiling one hour with 5% alcoholic potassium hydroxide. The reaction mixture was poured into water and extracted with ethyl ether. The ether solution was washed with water and evaporated to dryness on the steam-bath. The crude sterols obtained were dissolved in just sufficient boiling 85% ethanol to effect complete solution. After about twelve hours the crystalline material was separated, redissolved in a minimum amount of boiling 85% ethanol and let stand again. This procedure was repeated ten times. The resultant crystalline material was then recrystallized from methanol, and from chloroform–methanol; m. p. 168.5–169°; $[\alpha]^{27}D$ $-2.7°$ (556 mg., 10 cc. chloroform, l = 2 dm., $\alpha^{27}D$ $-0.30°$, *average* reading).

α-Spinasteryl Acetate.—A quantity of α-spinasterol was dissolved in acetic anhydride and the mixture heated one hour. On standing the crystalline acetate separated. The product was filtered off and recrystallized from 95% ethanol; m. p. 180–182°; $[\alpha]^{21}D$ $-6.4°$ (52.9 mg., 2 cc. chloroform, l = 2 dm., $\alpha^{21}D$ $-0.34°$, *average* reading).

α-Spinasteryl Benzoate.—Five hundred milligrams of α-spinasterol was dissolved in 1.5 cc. of pyridine and 0.5 cc. of benzoyl chloride added. The mixture was heated in a boiling water-bath two hours and allowed to stand twelve hours at room temperature. The product was recovered and recrystallized twice from 95% ethanol; yield 350

(4) Kuwada and Yosiki, *J. Pharm. Soc. Japan*, **60**, 161 (1940).
(5) Kuwada and Yosiki, *ibid.*, **57**, 155 (1937).
(6) Kuwada and Yosiki, *ibid.*, **59**, 282 (1939).
(7) Bernstein, Kauzmann and Wallis, *J. Org. Chem.*, **6**, 319 (1941).

(8) A small amount of water greatly facilitates the separation of the sterols from the oily mixture. These sterols tend to crystallize with one-half mole of water, if water is available.

mg.; m. p. 196–199°; $[\alpha]^{19}D$ 2.1° (51.6 mg., 2 cc. chloroform, l = 2 dm., $\alpha^{19}D$ 0.11°, *average* reading).

β-Spinasterol.—The combined mother liquors from the isolation of α-spinasterol were evaporated to a small volume and water added. The precipitate was filtered off and taken up in just enough boiling 85% ethanol to effect solution. After standing overnight at room temperature the solid material was filtered off. From the mother liquors a fraction, corresponding to the β-spinasterol of Heyl and Larsen,[2] was isolated. On recrystallization from 95% ethanol, flaky transparent crystals appeared; m. p. 148–150°. In melting they lost water of crystallization at 110–125°; $[\alpha]^{20}D$ 5.9° (52.7 mg., 2 cc. chloroform, l = 2 dm., $\alpha^{20}D$ 0.31°, *average* reading). The substance formed an insoluble precipitate with digitonin.

Anal. Calcd. for $C_{29}H_{47}OH \cdot {}^1/_2H_2O$: C, 82.58; H, 11.72. Found: C, 82.56; H, 11.98.

Anhydrous β-Spinasterol.—β-Spinasterol as isolated above was heated at 50° *in vacuo* for seven days. The product was free of water of crystallization; m. p. 148–150°.

Anal. Calcd. for $C_{29}H_{47}OH$: C, 84.38; H, 11.73. Found: C, 84.35; H, 12.12.

β-Spinasteryl Acetate.—One hundred milligrams of β-spinasterol was dissolved in acetic anhydride and the mixture heated one hour. On standing several hours at room temperature the crystalline acetate separated. The product was filtered off and recrystallized from 95% ethanol; m. p. 153–155°; $[\alpha]^{19}D$ 5.1° (44.7 mg., 2 cc. chloroform, l = 2 dm., $\alpha^{19}D$ 0.23°, *average* reading).

Anal. Calcd. for $C_{31}H_{50}O_2$: C, 81.88; H, 11.097. Found: C, 81.83, 81.54; H, 10.92, 11.38.

β-Spinasteryl Benzoate.—Five hundred milligrams of β-spinasterol in 1.5 cc. of pyridine was treated with 0.5 cc. of benzoyl chloride. The mixture was heated two hours on a boiling water-bath, allowed to stand overnight, and then handled as usual. The product was recrystallized from a mixture of ethyl ether and ethanol; yield 450 mg.; m. p. 181–183°; $[\alpha]^{19}D$ 7.5° (56.0 mg., 2 cc. chloroform, l = 2 dm., $\alpha^{19}D$ 0.42°, *average* reading).

Anal. Calcd. for $C_{36}H_{52}O_2$: C, 83.65; H, 10.15. Found: C, 83.81; H, 10.25.

Hydrogenation of β-Spinasteryl Acetate.—Nine hundred and fifty milligrams of β-spinasteryl acetate was dissolved in 25 cc. of glacial acetic acid and shaken for two hours in an atmosphere of hydrogen while in the presence of 100 mg. of Adams catalyst.[9] An additional portion of catalyst was added and the reaction continued about two hours more. The reaction mixture was filtered and the filtrate diluted with water. The product was then separated off and taken up in ethyl ether. The ether solution was washed with water, and the solvent removed by evaporation on the steam-bath. The product was recrystallized from 95% ethanol; yield 625 mg.; m. p. 115–116°; $[\alpha]^{17}D$ 9.6° (53.1 mg., 2 cc. chloroform, l = 2 dm., $\alpha^{17}D$ 0.51°, *average* reading).

Anal. Calcd. for $C_{31}H_{52}O_2$: C, 81.50; H, 11.48. Found: C, 81.90; H, 11.64.

This compound gave no depression in melting point when mixed with an authentic specimen of α-stigmastenyl acetate (prepared from authentic α-spinasterol).[10]

α-Spinastenol (α-Stigmastenol).[3]—The acetate mentioned above was hydrolyzed with 5% alcoholic potassium hydroxide. The product was recovered as usual and recrystallized from methanol; m. p. 111–112°; $[\alpha]^{18}D$ 21.2° (53.7 mg., 2 cc. chloroform, l = 2 dm., $\alpha^{18}D$ 1.14°, *average* reading).

Anal. Calcd. for $C_{29}H_{49}OH$: C, 83.97; H, 12.13. Found: C, 84.05; H, 12.28.

There was no depression in melting point when a specimen of this substance was mixed with authentic α-stigmastenol.

4. Fractionation of the Material Soluble in Cold Acetic Anhydride.—The crude acetates (Fraction A_2), m. p. 122–127°, were hydrolyzed with 5% alcoholic potassium hydroxide. The reaction mixture was poured into water and the product extracted with ethyl ether. The ether soluton was evaporated to dryness and the residue fractionally crystallized from ethanol. The more soluble fractions yielded a crystalline substance; m. p. 122–125°; $[\alpha]^{20.5}D$ −4.8° (48.7 mg., 2 cc. chloroform, l = 2 dcm., $\alpha^{20.5}D$ −0.23°, *average* reading). This material was not further studied.

δ-Spinasterol.—The less soluble top fractions from the above fractionation gave a product with a melting point of 142–143°. This substance, after repeated crystallization from methanol, gave a substance which appeared to be a chemical individual; m. p. 143–144°; $[\alpha]^{19}D$ 6.2° (49.6 mg., 2 cc. chloroform, l = 2 dm., $\alpha^{19}D$ 0.31°, *average* reading).

Anal. Calcd. for $C_{29}H_{47}OH \cdot {}^1/_2H_2O$: C, 82.58; H, 11.72. Found: C, 82.80, 82.33; H, 12.03, 11.94.

This substance formed an insoluble precipitate with digitonin and gave a positive Liebermann-Burchard test.

δ-Spinasteryl Acetate.—The sterol, m. p. 143–145°, was dissolved in a small amount of acetic anhydride and heated one hour. On standing at room temperature the acetate crystallized out. It was recrystallized from 95% ethanol; m. p. 132–133.5°; $[\alpha]^{16}D$ 0.8° (49.7 mg., 2 cc. chloroform, l = 2 dm., $\alpha^{16}D$ 0.04°, *average* reading).

Anal. Calcd. for $C_{31}H_{50}O_2$: C, 81.88; H, 11.097. Found: C, 81.42, 81.74; H, 11.30, 11.54.

This acetate on hydrolysis gave the sterol; m. p. 143–145°.

δ-Spinasteryl Benzoate.—Two hundred and ninety milligrams of the sterol was dissolved in 1 cc. of pyridine and treated with 0.5 cc. of benzoyl chloride. The mixture was heated two hours on a boiling water-bath and allowed to stand overnight at room temperature. The product was recovered as usual, and recrystallized four times from ethanol; m. p. 165–168° (softening gradually to a viscous liquid); $[\alpha]^{19}D$ 11.1° (50.4 mg., 2 cc. chloroform, l = 2 dm., $\alpha^{19}D$ 0.56°, *average* reading).

Anal. Calcd. for $C_{36}H_{52}O_2$: C, 83.65; H, 10.15. Found: C, 83.20; H, 10.37.

Hydrolysis with 5% alcoholic potassium hydroxide gave the original sterol, m. p. 143–145°, and the sterol recovered from the benzoate was converted to the acetate; m. p. 131–134°.

(9) Gilman and Blatt, "Organic Syntheses," Collective Volume 1, 2nd ed., John Wiley and Sons, Inc., New York, N. Y., page 463.

(10) We are indebted to Dr. H. G. Kolloff of the Upjohn Research Laboratories who very kindly supplied us with a specimen of α-spinasterol.

Hydrogenation of δ-Spinasteryl Acetate.—One hundred milligrams of δ-spinasteryl acetate was dissolved in 10 cc. of glacial acetic acid and shaken for one hour in an atmosphere of hydrogen in the presence of 50 mg. of Adams catalyst. The product was recovered and recrystallized twice from 95% ethanol; yield 40 mg.; m. p. 111–112°; $[\alpha]^{24}D$ 8.6° (44.5 mg., 2 cc. chloroform, l = 2 dm., $\alpha^{24}D$ 0.38°, *average* reading). This product when mixed with an authentic specimen of α-stigmastenyl acetate showed no depression in melting point.

Summary

1. From the unsaponifiable portion of Hardigan alfalfa seed oil we have isolated three isomeric sterols of formula $C_{29}H_{48}O \cdot {}^1/_2H_2O$: namely, α-spinasterol, β-spinasterol and a new sterol which was designated as δ-spinasterol.

2. Several derivatives of each of the sterols have been prepared and the physical constants and analysis observed.

3. Each of the three isomeric sterols can be reduced to α-stigmastenol.

EAST LANSING, MICHIGAN RECEIVED JULY 2, 1942

[CONTRIBUTION FROM THE DEPARTMENT FOR INORGANIC AND ANALYTICAL CHEMISTRY OF THE HEBREW UNIVERSITY]

Catalysts for Peroxide Decomposition[1]

BY M. BOBTELSKY AND A. E. SIMCHEN

Introduction.—In a previous paper[2] some facts were given relating to the catalytic decomposition of hydrogen peroxide in the presence of complex cobalt citrates acting as catalysts. One of them, pink in color, is stable and without catalytic activity; in the presence of hydrogen peroxide it is transformed into a green complex and simultaneously oxygen is evolved by the decomposition of the hydrogen peroxide. Gasometric experiments showed that the velocity of this evolution depends on the velocity with which the green complex is produced.

Nature of the Cobalt Citrate Complexes.—Our first concern was to ascertain the nature of the cobalt citrate complexes. This was accomplished by means of conductometric titrations of solutions of mono-, di- and tri-sodium citrates containing cobaltous ions.

These titrations were carried out with a Lautenschläger "Lyograph," in a cell of constant 0.52 with platinized and calcined platinum electrodes at 15 and 30°. The solutions of mono- and di-sodium citrates were prepared by mixing solutions of tri-sodium citrate and citric acid.

Figures 1 and 2 give some of our results. The breaks in curves 3 and 4 (Fig. 1) at 0.5 cc. of 1 M solution prove the presence of a compound with $Co^{++}/Ci^{---} = 1$. The small slope before the break indicates formation of undissociated molecules. Qualitative tests show that sodium hydroxide has no effect on the cobalt in these solutions. Figure 2 shows breaks in curves 8 and 9 at 1.5 cc. of 1 M NaH_2Ci and 0.75 cc. of 1 M Na_2HCi. These results prove that *only tertiary Ci^{---} ions take part in the formation of the complexes.*

The Catalytic Decomposition of Hydrogen Peroxide in Presence of Cobalto-citrate Complexes.—There are two phenomena to be elucidated: the transformation of the pink into the green complex and the catalytic decomposition of the hydrogen peroxide.

There are several possibilities as to the mechanism of the transformation of the pink complex. Hydrogen peroxide may not take part in an oxidation–reduction reaction, since experiments prove that cobalt is divalent in the green complex too (see below). Another possibility is that a peroxidized compound may be temporarily produced and then transposed into the green form.

The catalytic decomposition to hydrogen peroxide may also take place in several ways: hydrogen peroxide may add to the green complex forming an unstable addition compound; or oxygen atoms may add to the green complex and the resultant compound decomposes later, or the divalent cobalt may be oxidized to a higher valence and then immediately be reduced to Co^{++} with evolution of oxygen.

To test the various possibilities the quantities of two of the reactants, Co^{++}, Ci^{---} and hydrogen peroxide were kept constant and that of the third reactant was varied and the resultant changes in the system were measured by different

(1) An extensive revision of the original manuscript, chiefly concerned, however, with the presentation, was required and this was carried out under the Editor's direction. Under the disturbed international conditions now prevailing, it appeared impractical to obtain the authors' approval of this revision, and, to avoid delay, this revised version is therefore published on the Editor's responsibility.—THE EDITOR.

(2) M. Bobtelsky and M. Rappoport, *Compt. rend.*, **205**, 234 (1937).