or when several samples are used to cover the range of concentration in samples to be analyzed;
3. show procedure to be followed in B. Analysis of Data, and C. Example, when multiple samples are employed as in (2) above;
4. show in C. Example, how "agreement within (and between) laboratories" is determined. (Calculation of Confidence Limits or "T-Test");
5. add, under "*References,*" the textbook, "Introduction to Statistics," by Dixon and Massey.

The Statistics Committee has offered its services to any technical committee chairman in setting up an experimental design for collaborative work in determining the precision of an analytical method. They will assist also in the analysis of the analytical data obtained.

The Uniform Methods Committee has no doubt that many changes will be made during future months in this Section of Methods for "Determination of Precision and Accuracy of Test Methods." Its adoption as a Tentative Method is recommended. *Adopted.*

The Uniform Methods Committee has voted to request the Fat Analysis Committee to explore the need for methods of analysis for nitrogen derivatives of fatty acids and, if the need is found to exist, to take appropriate action for the development and adoption of such methods as are found to be essential.

As you can see, your technical committees have been busy and their progress reports indicate that in the future there will be continued activity in the formulation of new, and the improvement of present analytical methods.

The Uniform Methods Committee wishes to thank all our technical committee chairmen and the members of their committees who have made this progress possible.

J. J. GANUCHEAU
D. L. HENRY
T. H. HOPPER
K. E. HOLT
R. J. HOULE
T. C. SMITH
J. T. R. ANDREWS, chairman
E. M. SALLEE, editor of Methods

Pharmaceutical-Grade Sterols from Tall Oil[1]

CHARLES S. STEINER and EARLE FRITZ, Swift and Company, Chicago, Illinois

DURING RECENT YEARS there has been an increasing emphasis on medical research in the field of arteriosclerosis, popularly known as hardening of the arteries, and related disorders. Atherosclerosis, one form of arteriosclerosis, is characterized by the deposition of fatty matter on the inner walls of arteries. Atherosclerosis is the major factor in coronary artery disease and cerebrovascular accidents, popularly known as strokes. Although many theories have been advanced, the mechanism of the deposition of this fatty matter is still conjecture.

It is generally agreed among the medical profession that cholesterol, the predominant sterol found in animals, plays an important part since cholesterol is a major component of atherosclerotic deposits. Evidences have been produced which show that persons with atherosclerosis and related diseases may have a higher blood serum cholesterol content, known as hypercholesteremia, than those persons apparently free of artery disease (1). A reduction in cholesterol serum levels for hypercholesteremia patients appears desirable. However cholesterol is a very necessary ingredient for proper bodily functions. The body receives its cholesterol from two sources. Cholesterol is synthesized by the body and is absorbed from food sources found in a well-balanced diet. A lowering of serum cholesterol level sometimes can be attained by strict diet. Dieting is undesirable for the required type of diet is monotonous and unpalatable by American standards.

An oral intake of sitosterols, sterols found in vegetable and fruit sources, can be effective in reducing the level of serum cholesterol in patients with high cholesterol levels (2). The mechanism that causes this reduction is not known. One theory uses as an explanation that sitosterols interfere with absorption of cholesterol, possibly by the formation in the intestinal tract of a mixed crystal of sitosterol and cholesterol whose solubility is considerably less than that of cholesterol alone (3, 4). Other theories of the mechanism have been advanced. Figure 1 shows the chemical similarity of cholesterol, betasitosterol, and dihydrositosterol.

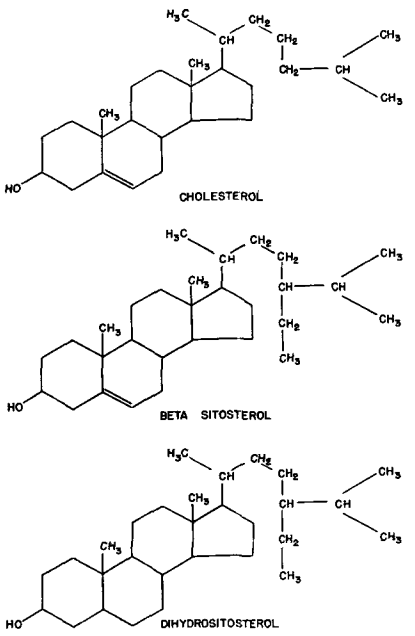

FIG. 1.

A commercial preparation of sitosterols for oral intake has been introduced. This preparation is a 20% suspension of betasitosterol and dihydrositosterol. The introduction of this therapeutic agent to lower serum cholesterol levels became possible when commercial quantities of suitable sitosterols made from tall oil were offered by Swift and Company. Betasitosterol, which seems to be the desired sterol for this application, is found widely distributed in vegetable sources. Particularly good sources for betasitosterol are tall oil and cottonseed oil. The composition of the sterols in these oils is 80% to 85% betasitosterol, 15% to 20% dihydrositosterol, and minor amounts of other sterols.

[1] This paper won first place in the 1958–59 Tall Oil Award of the Tall Oil Division of the Pulp Chemicals Association.

Sterols are concentrated in the foots in the alkali refining of vegetable oils. They are further concentrated upon distillation of acidified foots. Still bottoms from the distillation of cottonseed foots contain 7% to 10% total sterols. The sterol content varies quite widely depending upon the source, the refining, and distillation of the foots.

TALL OIL PITCH, resulting from the distillation of crude tall oil, offers the richest source of beta-sitosterols. Production of crude tall oil had reached 550,000,000 lbs. in 1955 (5) and production has increased continually. Of the crude tall oil produced and distilled in the United States, 15% to 25% results in pitch. Tall oil pitch is an inexpensive commodity and, in general, is in oversupply.

It may be conservatively estimated that the potential demand for sterols as a therapeutic agent for atherosclerosis could reach volumes requiring well over 50,000,000 lbs. of tall oil pitch per year. This assumes widespread acceptance by the medical profession and subsequent consumption by several million Americans who could derive some benefit from its use.

TABLE I

Typical Tall Oil Pitch Analysis

Color	Black
Acid number	65
Saponification number	110
Iodine number	140
Rosin acids	30%
Fatty acids	35%
Unsaponifiable matter	32%
Ash	0.05%
Softening point	90°F.

A typical analysis of tall oil pitch is shown in Table I. The composition of tall oil will vary considerably among producers. Since the primary concern was for the sterol content of tall oil pitch from different suppliers, samples from a number of suppliers were analyzed for unsaponifiable matter and total sterol content with results as shown in Table II. Satisfactory sterols could be made from each of the several sources listed, but yields drop with lower initial sterol content.

TABLE II

Unsaponifiable Matter and Total Sterol Content of Tall Oil Pitches

Source	Unsaponifiable matter	Total sterols
	%	%
1	32.0	16.0
2	26.0	12.0
3	27.3	10.5
4	31.0	6.8

The literature reveals a number of methods for the recovery of sterols (6, 7, 8, 9). Essentially these processes resort to saponification of the sterol source and extraction of the unsaponifiable matter with a suitable solvent. The unsaponifiable matter is recovered and dissolved in another solvent and crystallized therefrom. Usually one or more recrystallizations are necessary to produce a relatively pure product. Hickman (10) resorted to molecular distillation for purification. Generally the processes disclosed were found to be inadequate. Sterols produced by the foregoing methods do not meet the specifications, Table III, particularly on color and taste for pharmaceutical-grade sterols.

TABLE III

Pharmaceutical-Grade Tall Oil Sterol Specifications

Chloroform-insoluble	0.1% max.
Dirt	10 ppm. max.
Color (opt. den. @ 400 mμ)	0.100 max.
Melting point	134 to 140°C.
Specific rotation	−25 to −38°C.
Moisture and volatile	2% max.
Ash	1% max.
Heavy metals	20 ppm. max.
Sodium	0.1% max.
Methanol	0.0 ppm.
Total sterols [a]	95% min.
Unsaturated sterols	85% min.
Taste	Bland
Particle size	1-in. diam. max.

[a] By digitonin assay.

After an intensive laboratory and pilot-plant investigation, processing facilities to produce commercial quantities of pharmaceutically-pure sterols were installed at Swift and Company's Technical Products Plant at Hammond, Ind. Figure 2 shows a flow diagram of this process (11).

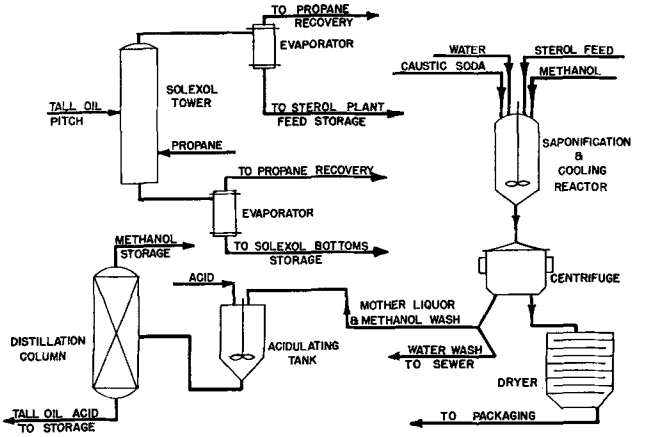

FIG. 2. Flow diagram. Pharmaceutical grade sterols from tall oil pitch.

The first step in the process, that of fractionation or extraction using liquid propane (Solexol process) (12), results in a light-colored (11C FAC) liquid overhead fraction. This overhead fraction contains 22% to 25% total sterols plus higher alcohols, fatty and rosin acids, and esters of fatty acids. The bottoms fraction has a ring and ball-softening point at 115°F. and consists principally of the oxidized polymerized components of the tall oil pitch. It has been found to have advantages for use in the same general areas that tall oil pitch has found application.

To accomplish the above fractionation the tall oil pitch is fed to the Solexol tower at a ratio of 20:1, solvent to pitch, at a tower temperature of 180°F. and a pressure of 680 psig. The fractionation results in approximately 50% each of overhead and bottoms. The composition of the tall oil feed-stock may necessitate changes in operating conditions.

The removal of the oxidized and polymerized components from tall oil pitch is necessary for the production of pharmaceutically-pure sterols. The omission of the propane fractionation results in a product containing unknown ingredients which impart undesirable color and taste to the final sterol product. In addition, these unknown ingredients inhibit proper crystal formation and growth. This results in impure sterols and lowers the rate of production.

The sterol content of the overhead fraction can be further increased to 30% to 35% by a conventional

FIG. 3. Sterols resulting from proper crystallization technique. 200 ×.

wet-refining procedure. This step, though desirable, is not essential.

Approximately half of the sterols in the overhead fraction are esterified with fatty acids. The next step is the saponification of the overhead fraction with 100% excess over theoretical caustic soda in methanol. Two volumes of 95% methanol are used for each volume of pitch overhead. Saponification occurs in 3 hrs. at gentle refluxing conditions. Hot water (160° F.) is slowly sprayed onto the surface of the batch until the volume is increased by 20%. Gentle refluxing is continued for another 2 hrs. The mixture is then cooled in 2 hrs. from 160°F. to 135°F. Gentle agitation is used throughout the saponification and chilling cycles. Care must be exercised to avoid sudden "shocking" of the batch either with cold reflux or with 20% water addition. If this care is not exercised, some of the soft and gummy unsaponifiable matter other than sterols precipitates. In addition, undesirable small crystals will result. The soft gummy material and the small crystals cause poor or negligible centrifuging and washing rates.

After chilling, the sterols are separated by using a perforated basket-type of centrifuge. When sufficient cake has been deposited, hot (140°F.) 95% methanol is sprayed into the cake and is continued until the effluent is colorless. Following the methanol wash, the cake is sprayed with hot water (180°F.) until the pH of the effluent is neutral. About 100 gals. of methanol and 100 gals. of water are required to wash 100 lbs. of sterols.

CONSIDERABLE experience and skill are required in the sequence of operations from the saponification to the final water-washing steps. With poor practice, precipitation of gummy material and small crystal formation result, and the resultant sterols are inadequate with regard to taste and color. The upgrading of a bad batch of sterols necessitates dissolving and recrystallizing in a solvent in which the sterols have an appreciably higher solubility than in methanol. Figures 3 and 4 show the difference in appearance of sterol crystals that result from proper and improper crystallization techniques.

After adequate washing the wet sterols are pelletized by using a modified meat grinder, placed on trays, and dried at 200°F. The dried sterols are packaged in fiber drums with a polyethylene inner lining.

The effluents from the centrifuge, through and including the methanol wash, are combined and then acidified. The methanol is recovered in a distillation column designed to produce 95% methanol overhead fraction. The still bottoms, excluding the water, has approximately 7–12% sterols, 25–30% other unsaponifiable matter, and 58–68% fatty and rosin acids, and a color of 37 FAC. Since these tall oil acids, containing a high percentage of unsaponifiable matter, are essentially free of oxidized and polymerized components, they may be distilled or used as a replacement for low-grade fatty acids.

The commercial plant was designed to produce 1,000 lbs. of sterols per day, requiring 11,000 lbs. of tall oil pitch per day. By-products amount to 5,500 lbs. per day of Solexol bottoms and 4,500 lbs. per day of tall oil acids with high unsaponifiable matter. Figure 5 shows a material balance of the process. Since the Solexol unit has a capacity considerably larger than required for the sterol plant, the Solexol

FIG. 4. Sterols resulting from improper crystallization technique. 200 ×.

unit in one day can produce a week's supply of feed material for the sterol plant.

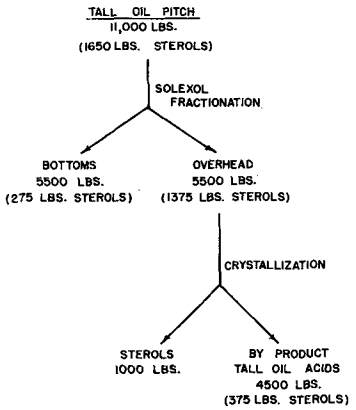

FIG. 5. Material balance.

With few exceptions all processing equipment is stainless steel. The acidulating tank is Monel, and the acid and caustic storage tanks are black iron. Every precaution is taken to remove dirt from the Solexol overhead fraction, methanol, caustic, and water used to charge the saponification reactor. Each of the charge lines for the above four streams is equipped with a filter. Once the overhead fraction and methanol come into contact, some of the sterols drop out of solution. Removal of dirt from this stage on becomes difficult. However magnetic particles are removed by a magnetic separator installed in the slurry line feeding the centrifuge.

The process described above uses a low cost by-product of the tall oil industry and has up-graded its value considerably. From tall oil pitch, pharmaceutical-grade sterols have been made. Thus a new commercial product has been added to the long list of products of the tall oil industry. Other large uses for tall oil sterols may develop, resulting in an increased demand for tall oil pitch. Considerable research is being conducted in steroid chemistry, such as cortisone, sex hormones, and many related compounds. The starting material for another medical triumph could be tall oil sterols.

Summary

A process has been developed for producing pharmaceutically-pure sterols from tall oil pitch. The process consists of the propane fractionation of the pitch, saponification of the overhead fraction in methanol, fractional crystallization, centrifuging, washing, and drying. A plant to produce 1,000 lbs. of sterols per day was brought into operation.

Acknowledgments

The authors gratefully acknowledge the help and encouragement given by H. C. Black, F. E. Lacey, and R. F. Thompson, and other personnel of the Research Laboratories and Technical Products Plant of Swift and Company.

REFERENCES

1. Page, I. H., Circulation, *10*, 1 (1954).
2. Lesesne, J. M., Castor, C. W., and Hoobler, S. W., Univ. Michigan, M. Bull., *21*, 13 (1955).
3. Peterson, D. W., Proc. Soc. Exper. Biol. and Med., *78*, 143 (1951).
4. Pollak, O. J., Circulation, *7*, 702 (1953).
5. Statistical Abstract of the United States, U. S. Department of Commerce, Bureau of the Census, 1956, p. 683.
6. Kruse, N. F., *et al.*, U. S. Patent No. 2,296,794.
7. Christenson, R. M., U. S. Patent No. 2,679,503.
8. Mattikow, Morris, *et al.*, U. S. Patent No. 2,704,764.
9. Albrecht, H., *et al.*, U. S. Patent No. 2,715,638.
10. Hickman, K. C. D., U. S. Patent No. 2,146,894.
11. Steiner, C. S., *et al.*, U. S. Patent No. 2,835,682.
12. Hixson, A. W., *et al.*, U. S. Patent No. 2,247,496.

[Received January 16, 1959]

ABSTRACTS . . . . R. A. REINERS, Editor

ABSTRACTORS: Lenore Petschaft Africk, R. R. Allen, S. S. Chang, Sini'tiro Kawamura, F. A. Kummerow, E. G. Perkins, and Dorothy M. Rathmann

• Fats and Oils

WAXES, THE NATURAL SURFACE COATINGS. H. H. Hatt (Commonwealth Sci. Ind. Research Organization, Melbourne). *Australian J. Sci.* 21, 31–42 (1958). An address. (*C.A.* 53,8665)

COMPOSITION OF THE PHOSPHOLIPIDE FRACTION OF BUTTER WITH SPECIAL REGARD TO THE POLYENOIC FATTY ACIDS. A. Deutsch, S. Mattsson, and P. Swartling. *Milk Dairy Research (Alnarp) Rept.* No 54, 9 (1958). Butter is shown to contain 1.7–2.5 g. of phospholipides/kg. The phospholipide fraction consists of about 38 mole % phosphatidyl-choline and 23 mole % sphingomyelin. One or more unidentified nitrogen-containing compounds among these, probably cerebrosides, are admixed with or attached to the phospholipides. The phospholipides contain substantially more unsaturated fatty acids than the corresponding butter triglycerides. Butter phospholipides contain approximately 4.7% dienoic, 2.5% trienoic, 1.2% tetraenoic, and 0.6% pentaenoic acid. (*C.A.* 53, 8468)

HIGH-ALLYL MUSTARD-RAPE HYBRID. V. I. Shpota. Maslobo˘ino-Zhirovaya Prom. 25(1), 20–2 (1959). *Brassica iuncea* x *B. napus* var. *oleifera* seeds of high-allyl oil content are described. (*C.A.* 53, 8665)

DEHYDRATION OF CASTOR OIL WITH SUBSTITUTED MONO- AND DISULFONIC ACIDS. A. S. Rajadhyakasha and K. K. Dole (Ferguson Coll., Poona). *Indian J. Appl. Chem.* 21, 95–8 (1958). The efficiency of a number of substituted sulfonic acids as catalysts in the dehydration of castor oil was reported. When a molar concentration of catalyst was used, the order of activity was chloro-> bromo-> iodo> unsubstituted benzenesulfonic acid. Dark oils were obtained; 2-naphthol-6,8-disulfonic acid produced least color. Dehydration at low pressure improved color. (*C.A.* 53, 9698)

FRACTIONATION AND PREPARATION OF FATTY ACIDS AND ESTERS FROM NATURAL MIXTURES WITH THE HELP OF UREA COMPLEXES. S. Adhikari and N. A. Khan (Pakistan Council Sci. and Ind. Research, Tejgaon, Dacca). *Pakistan J. Sci. Research* 1, 221–3 (1958). The methyl esters of cottonseed, linseed, and castor oil were fractionated as urea complexes. Acids of sesame and mustard oil gave good separation, but some esterification occurred when methyl alcohol was used as a solvent. (*C.A.* 53, 9699)

DERIVATIVES AND ISOLATION OF PURE GOSSYPOL. K. Chander and T. R. Seshadri (Univ. Delhi). *J. Sci. Ind. Research* (India) 17B, 279–80 (1958). A simple method of extracting gossypol is based on the extraction of an ethereal extract of cottonseed with an aqueous solution of borax, removal of gossypol in the form of a soluble borate complex, and regeneration by treatment with acid. Pure samples of the hexa- and dimethyl ethers of gossypol can be obtained by methylation with methyl sulfate and potassium carbonate in acetone solu-