

(5) Paul S. Pittenger, "The Actions and Biologic Assay of Ephedrine," *JOUR. A. PH. A.*, 17 (1928), 634-640.

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## THE BELLIER TEST FOR SESAME OIL.\*

BY E. FULLERTON COOK.

Is the Bellier test infallable, is it absolutely distinctive for Sesame Oil, how small a percentage of sesame oil in other oils will it detect, what conditions interfere with its application, can the principle responsible for the Bellier reaction be removed and can the original test be improved?

These are some of the obvious questions presenting themselves in the undertaking of this investigation.

Sesame oil is of much greater importance in Europe than in the United States as sesame seed is largely cultivated there, and the oil which it supplies is a valuable food and technical product while cotton seed oil and corn oil of the United States are practically unknown in Europe, except as a museum curiosity. Furthermore, the food laws in some European countries require the presence of a small percentage of sesame oil in all "Oleomargarine" as a ready means of identification, hence distinctive tests are of importance.

The Bellier reaction was first noted in the literature in 1899<sup>1</sup> and is now one of the several tests commonly used to identify or detect the presence of sesame oil.

The usual directions for the test are:

Over 1 volume (about 1 cc.) of colorless nitric acid (sp. gr. 1.4), in a test-tube, carefully overlay 1 volume of the oil to be tested, then add 1 volume of benzol, containing 1.5 per cent of resorcinol and shake once. The acid strata should become intensely green if sesame oil is present (which is the characteristic feature of the test) and the benzol-oil strata becomes violet. These colors quickly fade to dark red.

Considerable difficulty was experienced when using this technique in always obtaining the distinctive green color even when using the same acid and oil and, with even the most exact following of the technique, the test was at times uncertain in its results.

For this reason modifications were studied and the following is recommended as more simple, far more reliable and more sensitive.

### MODIFIED BELLIER TEST (COOK).

Add 2 drops of the oil to 1 cc. of colorless nitric acid (sp. gr. 1.4) in a test-tube, agitate slightly, then add a small crystal of resorcin and again agitate. If sesame oil is present a green color should develop about the oil and extend into the acid. On adding benzol or chloroform practically all of the green color remains in the acid strata but fades within 3 to 5 minutes.

\* Scientific Section, *A. PH. A.*, Rapid City meeting, 1929.

<sup>1</sup> Experimental tests made in the Pharmaceutical Institute, University of Berne, Berne, Switzerland, under the direction of Prof. Alexander Tschirch.

*Ann. chim. anal. appl.*, 4 (1899), 217-220; *Jahresb. Chem.* (1899), 1176; *Chem.-Zig.* (1899), 263.

NOTE: If the crystal of resorcinol is too large the brown color resulting from the action of  $\text{HNO}_3$  on resorcinol will obscure the green color. Therefore, use a small crystal.

#### SENSITIVENESS OF MODIFIED BELLIER TEST (COOK).

After many experiments it was found that a 0.4 per cent (1 in 250) solution of sesame oil in peanut oil was the smallest amount which could be definitely detected by this modified test. It was also found that the Furfurol (Bandoninsche) Test would detect 5 per cent (1 in 20) of sesame oil in solution with peanut oil but no less.

#### DISTINCTIVE CHARACTER OF THE BELLIER TEST.

A number of commercial samples of seed oils were bought and tested for their response to the Bellier test and subsequently these same kinds of oils and some others were prepared by extraction and also tested by the Bellier reaction. All reactions were observed and the characteristics noted immediately and then after 10, 20, 30 and 60 seconds, and also after 2 and 5 minutes.

These details are not given as they contributed little to the study here reported.

#### RESULTS OF APPLYING THE ORIGINAL BELLIER TEST TO SEED OILS.

<i>Arachis (Peanut) Oil</i>	No. 1	(8 tests)	Negative
	No. 2	(3 tests)	Negative
<i>Linseed Oil</i>	Extracted	No. 15E (1 test)	Negative
	Extracted	No. 16E (1 test)	Negative
	No. 1	(2 tests)	Negative
	No. 3	(2 tests)	Negative
<i>Castor Oil</i>	Extracted	No. 2E (2 tests)	Negative
	Extracted	No. 10E (2 tests)	Negative
	No. 1	(2 tests)	Negative
	No. 3	(2 tests)	Negative
	Extracted	No. 3E (2 tests)	Negative
	Extracted	No. 4E (1 test)	Negative
<i>Olive Oil</i>	Extracted	No. 5E (1 test)	Negative
	Extracted	No. 6E (1 test)	Negative
	No. 1	(2 tests)	Negative
	No. 2	(2 tests)	Negative
<i>Rape Oil</i>	No. 3	(2 tests)	Negative
	No. 1	(2 tests)	Negative
	No. 2	(2 tests)	Negative
<i>Almond Oil</i>	Extracted	No. 1E (2 tests)	Negative
	Extracted	No. 9E (2 tests)	Negative
	No. 1	(2 tests)	Negative
	No. 2	(2 tests)	Negative
<i>Peach Kernel Oil</i>	Extracted	No. 11E (2 tests)	Negative
	Extracted	No. 12E (2 tests)	Negative
<i>Cotton Seed Oil</i>	No. 1	(2 tests)	Negative
	No. 2	(2 tests)	Negative

*Oil of Theobroma*.—No green color in response to the modified Bellier (Cook) test.

*Walnut Kernel Oil*.—No green color in response to the modified Bellier (Cook) test.

These tests were followed by the application of the "Modified Bellier (Cook) test" with a negative response by all oils but Sesame which invariably responded to the test (Note: The "No. 1 Sesame Oil," bought commercially, never responded to this test and was shown not to be sesame oil).

		SESAME OILS.														
Test No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
No. of Sesame Oil		1	2	2	2	2	2	3	3	3	4	4	13E*	13E	14E	14E
Vol. HNO <sub>3</sub>		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Vol. Sesame Oil		1	1	1/4	1	1/4	2	1	1	1/4	1	1	1	1	1	1
Resorcin-Benzol																
Vol. solution																
1.5%		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
With little shaking			✓	✓				✓		✓	✓		✓		✓	
With vigorous shaking		✓			✓	✓	✓		✓			✓		✓		✓
Result—no green color		+	+	+			+	+			+		+		+	+
No green color		-	-		-	-			-	-		-		-		

\* "E" indicates that the sample was obtained by extraction from the seed.

*Can the Principle Responsible for the Green Color of the Bellier Test for Sesame Oil Be Removed?*—The oil was subjected to many tests involving extraction, carbonization, saponification or enzymatic action and the only method found successful in removing the principle, causing the green color of the Bellier test, was repeated washing with glacial acetic acid, at least 10 washings being necessary to complete the extraction.

*Negative Result from the Following Treatments.*—Extractions were made with water, with 50, 60, 70 and 80 per cent alcohol, with 5, 10, 20 and 30 per cent hydrochloric acid, with 20 per cent sulphuric acid, with acetone, acetic ether, a mixture of acetone 4-alcohol 1, a mixture of equal volumes acetic ether and alcohol, a mixture of acetone 2-water 3, a mixture of acetone 4-water 1, a mixture of ether 1-water 2, a mixture of chloroform 1-water 2, a mixture of petroleum ether 1-water 2.

After all of these attempted extractions the separated sesame oil responded without change to the test.

The addition of 3 drops of 96% sulphuric acid to 10 cc. of sesame oil and subsequent heating to 150° C. did not lessen the response of the oil to the test.

On heating sesame oil and water, at boiling temperature for two hours and separating the oil it still responded to the test.

On treating sesame oil to the action of lipase (from a 10% emulsion of castor oil beans) at about 35° C., for 3 days, with frequent agitation, and then allowing to stand, the upper strata was much thickened and filled with crystalline masses of fatty acid. This crystalline mass and the separated oil still responded to the modified Bellier (Cook) test.

On heating 50 Gm. of sesame oil to exactly 250° C., withdrawing a sample and cooling immediately the Bellier test was normal. On continuing the 250° C. on the remainder of the oil for 30 minutes, the oil still responded normally to the Bellier test.

On treating the sesame oil with hot 50% sulphuric acid and also after passing live steam through the mixture, without pressure, for several hours the separated oil responded to the modified Bellier reaction without change.

On completely saponifying sesame oil with KOH in 70% alcohol, the resulting

soap responded to the modified test although the green color was slightly tinged with brown.

On decomposing the soap just described by the addition of hydrochloric acid, the separated fatty acids still responded to the test.

On shaking 50 Gm. of sesame oil with 5 Gm. of kieselguhr and filtering, the separated oil was positive to the Bellier test.

On treating sesame oil with Lloyd's reagent (freshly hydrated aluminum silicate) and filtering, the oil is still positive to the Bellier test.

On extracting the hulls of sesame seed with petroleum ether, the resulting extract was negative in its reaction to the modified Bellier (Cook) test.

*Positive Result from Extraction of Sesame Oil.*—On shaking out 100 cc. of sesame oil, not less than ten times, with 40 cc. of glacial acetic acid, the oil, thus extracted, no longer responded to the modified Bellier (Cook) test.

*The principle causing the green color of the Bellier reaction in sesame oil is not sesamin.* Villavechia and Fabris<sup>1</sup> gave a method for extracting a crystalline principle, which they called Sesamin, from sesame oil.

Their process was as follows:

Saponify the sesame oil with alcoholic KOH, evaporate the alcohol, dissolve the dried soap in water, warm the solution and precipitate with an excess of barium chloride. (This barium soap gave the green color to the modified Bellier test but the KCl solution did not.)

The barium soap is then dried, digested with boiling alcohol and the alcohol solution separated and evaporated. Crystals of sesamin separate which, according to V. and F., give no color reactions.

This was verified by the author.

Malagnini and Armauni<sup>2</sup> also reported a method for extracting a white crystalline body which they claim gives intense color reactions.

They reported the extraction of sesame oil with a mixture of petroleum ether 30 parts—alcohol 70 parts and the obtaining of a white crystalline body which gave intense color reactions.

In attempting to carry out this process it was evident that the mixture dissolved a portion of the oil and both strata gave an intense green to the modified test.

The petroleum-ether-alcohol strata was still further shaken out with petroleum ether to remove dissolved oil and again separated. Both strata still give the Bellier reaction the alcoholic one, apparently most intensively.

On evaporating the alcohol strata it yielded a few drops of oil and a minute amount of a seemingly crystalline substance.

This oil and crystalline mixture was washed with petroleum ether to remove oil. The washings gave the Bellier reaction but no satisfactory crystalline principle was obtained.

*Extraction with Glacial Acetic Acid.*—Having observed that repeated washing (not less than 10) with glacial acetic acid removed from sesame oil the principle responsible for the green color of the Bellier test, an effort was made to separate this principle from the Acetic Acid extract as follows:

The combined acetic acid washings were shaken out with ether and the combined ether extracts were evaporated spontaneously. The resulting residue con-

<sup>1</sup> *Jahresbericht d. Pharm., abst.* (1897), 708.

<sup>2</sup> *Jahresbericht d. Pharm.* (1907), 439.

sisted of an upper oily layer and a lower brown-colored strata which was strongly acid.

This residue was then treated with petroleum ether to separate the oil and the oil was discarded.

The remaining brown-acid extract was again taken up with ether and the solution evaporated spontaneously. The residue consisted of a brown, crystalline mass. This residue was then dissolved in 95% alcohol (it was not entirely soluble or at least not all readily soluble) and filtered. This alcoholic solution, on evaporation, left a residue containing small white crystals in the center and a brown resin-like mass on the outer edge. The brown resin-like residue gave the Bellier reaction strongly.

The crystalline mass was again dissolved in ether (the crystals were hard and dissolved slowly) and allowed to evaporate spontaneously. The resulting crystals were almost colorless, well-formed, rhombic and corresponding in most physical factors with the reported characteristics of sesamin. These crystals did not give a color reaction to the Bellier test and the assumption is that the principle causing this color reaction will be found in the resin-like mass referred to above.

No further effort has been made to isolate it.

The following table shows the various physical factors determined for the crystalline substance extracted from sesame oil.

PHYSICAL FACTORS FOR SESAMIN.		
	Crystals obtained by glacial acetic acid extraction 121 to 122° C.	Sesamin as reported by Villavechia and Fabris— <i>Jahresbericht d. Pharm.</i> (1897), 708. 122° C.
Melting point.		
<i>Soluble</i> (leaving crystals on evaporation)	Alcohol (95%); absolute alcohol; ether (slowly); benzol (readily)	Alcohol (colorless needles); chloroform (prismatic crystals)
<i>Soluble</i> (leaving amorphous residue on evaporation)	Acetone (readily); acetic ether; chloroform (readily); glacial acetic acid	Benzol; glacial acetic acid
<i>Insoluble</i>	Cold and hot water; petroleum ether	Ether, water, petroleum ether
HNO <sub>3</sub> (1.4)	Green color	No color reactions
Furfurol test. (Bandoninche reaction)	Negative	
HNO <sub>3</sub> -Resorcin-cryst. reaction	Negative	

#### CONCLUSIONS.

The answers so far determined for the several questions propounded at the beginning of the paper are:

1. The modified Bellier (Cook) Test is invariable in its response on all sesame oils examined. The original Bellier test is uncertain.
2. This test is distinctive for sesame oil so far as oils for the test were available and this included all common fixed oils.
3. The modified test will detect 1 part of sesame oil in 250 parts of a dilution in another fixed oil.
4. Too much crystal resorcinol in the modified test and too vigorous or prolonged shaking in the original test interfere with the development of the green color.
5. So far as experiments were conducted they indicate that sesame oil can

only, with much expense and labor, be freed from the principle which causes this distinctive color reaction.

6. The modified test, as suggested by the author, is believed to be a distinct improvement over the original test, giving more assured results and a more sensitive test.

#### ADDITIONAL REFERENCES.

*Chem.-Ztg.* (1899), 263.

*Jahresbericht Pharm.* (1903), 525; (1906), 450; (1907), 439-440; (1908), 398-400; (1909), 475-476; (1910), 421; (1911), 367; (1912), 428; (1913), 364, 385, 390; (1918), 303; (1919), 327 and 959; (1921), 316 and 327; and (1922), 299.

*Jahresbericht Chem.* (1899), 1175, 1176, 1179, 1180; (1900), 749 and 750; (1901), 782, 783, 791, 792; (1902), 955; (1903), 934; (1904), 1073; (1905-1908), II, 572, 581, 582, 662; (1909), 235, 242 and 244; (1910), 248, 257, 259.

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## THE ACID-BASE EQUILIBRIUM OF TINCTURE OF DIGITALIS.\*

BY JOHN C. KRANTZ, JR.

### INTRODUCTION.

Recently a considerable amount of investigation has been carried out to study the effect of changes in hydrogen-ion concentration upon the stability of the various preparations of digitalis. Tainter (1) showed that the physiological activity of freshly prepared infusions of digitalis is independent of their hydrogen-ion concentrations, and that the infusion tended to become acid upon standing. This investigator determined the acidity of the tincture to be the equivalent of an  $N/10,000$  hydrochloric acid solution, approximately  $p_H$  4.6. Takahashi (2) contrary to the results of Tainter showed that the addition of 0.05 per cent to 0.1 per cent of hydrochloric acid to the infusion of digitalis increases the stability of the infusion as determined by the frog method.

Smith (3) in his studies of the determination of the hydrogen-ion concentration in alcoholic solutions investigated tincture of digitalis and found the range of  $p_H$  to be between 5.12 and 5.77. Of special interest, is the work of Joachimaglu and Bose (4) who showed the stability of tincture of digitalis to be increased by the addition of 0.1 or 0.2 per cent of tartaric acid. These investigators found the  $p_H$  of tincture of digitalis to be 5.88, with 0.1 per cent tartaric acid 5.44 and with 0.2 per cent of the acid 5.13.

With the thought of studying the influence of buffer-salt mixtures in the menstruum upon the stability of the tincture, this investigation was begun.

*Buffering the Menstruum of Tincture of Digitalis.*—A series of menstrua were prepared using an 80% alcohol and the citrate mixtures described by Sorensen (5). With these a series of tinctures were prepared by the U. S. P. method omitting the extraction of the drug with petroleum ether. The hydrogen-ion concentrations of the menstrua were determined by means of the hydrogen electrode (Wilson type (6)). The values obtained were reproducible within 0.1 unit  $p_H$ .

Table I indicates the composition of the buffer mixtures, the  $p_H$  of the menstruum and the  $p_H$  of the tincture.

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\* Scientific Section A. PH. A., Rapid City meeting, 1929.