Scientific Section

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THE ASSAY OF BALSAM OF PERU.*

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"Balsam of Peru" is the name applied to the resinous exudation obtained from the tropical tree, *Myroxylon Pereirae,* occurring in Central America, principally in the republic of San Salvador.

The balsam comes into commerce from the port of San Salvador; is of some medicinal importance, and also finds a limited application in perfumery.

As regards its chemical composition, the early investigations of Delafontaine,[1] Frémy,[2] and Kraut,[3] showed the presence of neutral aromatic esters (benzyl benzoate and cinnamate), amounting to over fifty percent. The acid resin, according to Kachler,[4] yielded about sixty percent of protocatechuic acid.

A more thorough examination of the balsam was made by Thoms,[5] who found the cinnamein (or neutral esters) to consist principally of benzyl benzoate and cinnamate, with possibly some hydrocinnamic ester.

He also isolated a new alcohol, peruviol, to which he assigned the formula, $C_{13}H_{22}O$. This compound, a light liquid with characteristic odor, was later found by Schimmel & Co.,[6] to be identical with nerolidol, $C_{15}H_{26}O$, the sesquiterpene alcohol isolated by Hesse and Zeitschel[7] from the high-boiling fractions of oil of orange flowers.

For the assay of the balsam, a rather large number of tests have been proposed, and are embodied in the various pharmacopœias and similar works. Those depending on color reactions are in general unreliable and of limited value. Dieterich[8] lays much stress on the acid value, saponification value, Cinnamein content, and saponification value of the latter; and the determination of these analytical constants is almost universally required by the best authorities. Yet it is obvious that such values are far from being absolutely characteristic: that they are insufficient to demonstrate the purity or authenticity of a sample is a fact unpleasantly brought to our attention by the appearance in recent years of imitation or "synthetic" balsams almost indistinguishable from the natural product.

* Read before Scientific Section, San Francisco Meeting.

[1] Zeitschr., 1869, 156.
[2] Ann. 30, 330.
[3] Ann. 152, 129.
[4] Ber. 2, 512.
[5] Arch. d. Pharm. 237, 271.
[6] Berichte, April, 1914.
[7] J. f. prak. Ch., 1902, 504.
[8] Analyse de Harze.

Evers[9] patented a mixture of styrax, esters, and gum resins, which was asserted to be fully identical with the natural balsam; and similar products, bearing the names "Perugen" and "Perugran" have become articles of commerce.

The Perfumery and Essential Oil Record, in a note on this subject,[10] remarks: "Samples submitted to us from M. Schultz & Co., Hamburg, are remarkable imitations of natural balsams, and in chemical and physical characters are almost indistinguishable from the genuine products." And as far as the pharmacopœial standards and usual tests go, we can only confirm this statement, as following analyses show.

No. 1 is an artificial product recently examined; No. 2 is an average sample of some thirteen lots, of direct importation, and, as far as we can ascertain, of undoubted authenticity.

	Sp. Gr. at 25°	Acid Value	Percent Cinnamein	Sap. Value of Cinnamein
No. 1	1.1542	59.68	62.3	254.9
No. 2	1.1535	65.20	54.1	235.9

Jensen[11] also gives figures showing close approximation in these constants.

Such "synthetic" products naturally become objects of suspicion as possible adulterants of the genuine and more expensive balsam, and their detection in mixtures becomes of some importance.

For this purpose the determination of the iodine value of the cinnamein, or neutral portion of the balsam, has been proposed. Jensen (l. c.) in examining three samples, one of which was imitation, found the iodine value of the natural cinnamein, 25.5; of the imitation, 1.5.

We found on Sample 1 (above) I.V. 3.6; on Sample 2, I.V. 21.0. Dieterich,[12] however, reported the analyses of various samples, in which the I.V. of the imitation cinnamein was found as high as 27.5. If these results are correct, it is evidently unsafe to put much reliance on this test, for the possibility is not excluded that substances may be added to the imitation product to increase its iodine value.

Benyzl benzoate shows no absorption of iodine: we found that pure benzyl cinnamate, obtained by freezing the natural cinnamein, gave an I.V. of about 7.0. Jensen (1. c.), on fractioning natural cinnamein in vacuo, found that the first 10% of the distillate had the I.V. 116, and we obtained a similar value for the crude peruviol, described below.

Optical activity is also characteristic of the natural cinnamein, and is due to the peruviol, which Thoms found to have $a_D = +13°$. The determination of the rotation of the crude cinnamein is, however, impracticable.

Peruviol, with its high iodine value, and dextro-rotation, appears to be the most characteristic constituent of the balsam, and after some experiment, the following simple method of isolating it was devised.

Peruviol Test.—Twenty gms. of balsam are saponified by heating one hour on the water-bath, with frequent shaking, in a liter flask, with 20 gms. of 25% potassium hydroxide. Steam is then passed through the mixture, and the dis-

[9] Pharm. Ztg. 49, 524.
[10] March, 1914, 86.
[11] Pharm. J., 1913, 210.
[12] Perf. and Ess. Oil Rec., 1914, 89.

tillate collected in 100 or 150 cc. flasks with narrow, graduated necks. From natural balsams, we obtained in this way, in 300 cc. total distillate, from 0.7 to 0.9 cc. of light oil. Imitation balsam gave only traces of heavy oil.

The crude peruviol from 100 gms. balsam amounted to 4.5 cc. and showed the Sp. Gr. 0.917 at 25°, a_D=10°. Thoms found for a purer product, Sp. Gr. 0.886 at 17.5°, a_D=13°. Our preparation contained evidently some benzyl alcohol, and, in fact, by saturating the aqueous distillate with salt, 8 cc. of oil were obtained, with Sp. Gr. 1.038 at 25°, inactive, and almost completely soluble in 40 volumes of water.

Natural cinnamein contains, then, about 4% of peruviol, and benzyl alcohol is practically the only other alcoholic constituent. From 100 gms. of imitation balsam, only benzyl alcohol (Sp. Gr. 1.042 at 25°, inactive) was obtained. For identification, the peruviol from 20 gms. of balsam is sufficient. It is a colorless oil, of characteristic odor, soluble in 70% alcohol, and showing an iodine value of about 116.

If 20 gms. of balsam are distilled in the manner described, without saponification, less than 0.1 cc. of light oil is obtained, with a small quantity of heavy oil (benzyl benzoate). This indicates that the peruviol is present mostly as ester in the balsam.

The following are typical analyses from our records:

	No. 30	No. 145	No. 192	No. 776
Sp. Gr. at 25.....	1.1462	1.507	1.154	1.154
Acid Value.....	67.3	71.0	72.8	67.6
Cinnamein	58.3%	54.6%	54.6	54.7
Sap. Val. of do.....	230.5	235.1	242.4	236.3
Peruviol (ex 20 gms.).....	0.9 cc.	0.9 cc.	0.65 cc.	0.85 cc.

The peruviol determination appears to be a very reliable indication of the quality of a balsam. Adulteration with fatty oil or rosin, would lower the yield of peruviol: if copaiba were present, the peruviol would be contaminated with the oil of copaiba, and would show a diminished solubility in alcohol, and a lower, or possibly a lævo-rotation.

ACID CONSTITUENTS OF PERU BALSAM.

Thoms (l. c.) found benzoic and cinnamic acids present in the ratio of about 3 to 2, using for the determination of the latter the oxidation method of Liebermann, which is based on the reaction:

$$C_6H_5CH{=}CH{-}COOH + O_5 = C_6H_5COOH + 2CO_2 + H_2O.$$

We found the acids in about the same proportion in the balsam, but in the imitation practically only benzoic acid.

The analyses were made in the following manner. Samples of natural and imitation cinnamein were saponified, the solutions evaporated to dryness, dissolved in water, extracted with ether, acidified, and the liberated acids separated and dried. Weighed amounts of acid were then dissolved in N/2 alkali, and the solutions diluted to contain 1 gm. in 100 cc. For each titration, 5 cc. were run into 25 cc. of N/2 permanganate; after standing one-half hour, the excess of permanganate was titrated in the usual manner with standard oxalic acid. For comparison, samples of benzoic and cinnamic acids were also titrated. According

to the above equation, one gm. cinnamic acid should require 135.1 cc. N/2 permanganate.

ASSAYS.

	(Time)	(cc. $KMnO_4$ per gm.)	(Percent Cinnamic Acid)
(1) Benzoic acid	½ hr.	6.0	4.4
(2) do	do	8.0	5.8
(3) Cinnamic acid	do	141.8	104.9
(4) do	do (warm)	157.8	116.8
(5) Ex balsam	½ hr.	57.0	42.1
(6) Ex imitation	do	6.0	4.4

These results were far from satisfactory; apparently the oxidation of cinnamic acid was not quite so simple as the equation indicated. In fact, when an acid permanganate solution was used, even more erratic values were obtained, e. g. (with permanganate containing 2% H_2SO_4).

(7) Cinnamic acid.....	5 min.	248.9 cc. $KMnO_4$ per gm.	
(8) do	20 min.	263.1	do
(9) Ex balsam.....	5 min.	119.4	do
(10) Ex imitation.....	5 min.	19.8	do

It was evident that to secure any useful values, a more careful modus operandi was necessary: it was soon found that better results were obtained when the oxidizing solution was gradually added.

(11) Cinn. acid.....	1. gm. required	142.3 cc.	
(12) do	0.1 gm. do	13.8 cc. (138.0 cc. per gm.)	
(13) do	0.1 gm. do	13.7 cc. (137.0 cc. per gm.)	(MgO)

With permanganate always in excess, the results were not uniform.

(14) Cinn. acid	.05 gm. req. 7.95 cc.	(25 cc. used)		(159 cc. per gm.)
(15) do	.1 gm. req. 14.6 cc.	do		(146 cc. per gm.)
(16) do	.1 gm. req. 14.4 cc.	do	(MgO)	(144 cc. per gm.)
(17) do	.1 gm. req. 14.9 cc.	do	(MgO)	(149 cc. per gm.)

In Nos. 13, 16, and 17, the solution contained $MgSO_4$ in the proportion: $2KMnO+MgSO_4$, to ensure constant neutrality.

With acid permanganate, the following values were found:

(18) Cinn. acid	.1 gm. req. 24 cc.	($KMnO_4$ added slowly)	(240 cc. per gm.)
(19) do	.05 gm. req. 13.9 cc.	(25 cc. used)	(278 cc. per gm.)
(20) do	.10 gm. req. 29.5 cc.	(45 cc. used)	(295 cc. per gm.)
(21) do	.10 gm. req. 28.7 cc.	(50 cc. used)	(287 cc. per gm.)
(22) do	.10 gm. req. 27.0 cc.	(75 cc. psed)	(270 cc. per gm.)

In Nos. 21 and 22, the mixtures were allowed to stand one hour before titrating back. The oxidizing solution contained sulphuric acid in the proportion: $2KMnO_4+3H_2SO_4$.

These results in acid solution were unexpected. Benzoic acid is supposed to be unaffected by the reagent under these conditions, and the slight reduction of permanganate which we observed must be due to impurities. For example:

(23) Benzoic acid	.10 gm. req. 0.5 cc.	(10 cc. used)	(5 cc. per gm.)
(24) do	.10 gm. req. 1.0 cc.	(25 cc. used)	(10 cc. per gm.)
(25) do	.10 gm. req. 1.2 cc.	(5 cc. acid $KMnO_4$)	(12 cc. per gm.)
(26) do	.10 gm. req. 0.7 cc.	(25 cc. used)	(7 cc. per gm.)
(27) do	.10 gm. req. 0.7 cc.	(25 cc. acid $KMnO_4$)	(7 cc. per gm.)

A solution of sodium benzoate was purified by heating several days with excess of permanganate; the latter was then reduced with a little bisulphite, and the solution diluted to contain one percent of acid.

(28) 10 cc. of this solution required 0.45 cc. (25 cc. used) (4.5 cc. per gm.).

A sample of acid prepared from the recrystallized calcium salt gave no better result, and the cause of this slight action on the permanganate remains unexplained.

In the case of cinnamic acid, it is remarkable that the values in acid solution are about twice the theoretical, approaching a limit even with a large excess of reagent (No. 21 and 22). It is not incredible that benzoic acid in the "nascent" condition may be more readily oxidized in acid solution, and a portion completely burnt to carbonic anhydride and water. But, if so, one would hardly expect any constancy in the amount of oxidizer used.

It appeared of interest to determine the actual amount of benzoic acid produced by oxidation in neutral and acid solutions, respectively.

(29) 10 cc. of a solution of sodium cinnamate containing one percent of the acid were run slowly into 25 cc. N/2 permanganate. After one hour, the excess of reagent was reduced by oxalic acid, and then the titration continued as usual with oxalic and sulphuric acids. Required: 14.3 cc. 143 cc. per gm.). The solution was then extracted with ether, and the ether solution evaporated to constant weight. The white crystalline acid weighed 0.076 gm. (calc. 0.082 gm.), and on titration required 6.2 cc. N/10 alkali, equivalent to 0.0756 gm. benzoic acid.

(30) 10 cc. of the cinnamic solution previously used were run into a mixture of 50 cc. permanganate and 15 cc. normal sulphuric acid. After one hour, titration as before. Required, 27.3 cc. (273 cc. per gm.). The acid taken out by ether, weighed 0.052 gm. and on titration, required 4.22 cc. N/10 alkali, equivalent to 0.0515 gm. benzoic acid.

In this last experiment, there is a deficiency in the yield of benzoic acid amounting to 0.030 gm. Assuming this to have been completely oxidized, according to the equation:

$$C_7H_6O_2+O_{15}=7CO_2+3H_2O$$

for 0.03 gm., 0.233 gm., or 14.7 cc. N/2 permanganate would be required, which is about the difference observed in the neutral and acid titrations on 0.10 gm. cinnamic acid. Hence it appears that on oxidation by permanganate, in acid solution, under the conditions described, about 36% of the cinnamic acid is completely oxidized to carbonic anhydride and water.

For the examination of the mixed acids from the balsam, however, the procedure of No. 11-13 will give useful results The presence of cinnamic acid is not, of course, conclusive evidence of the purity of the balsam, for the addition of a cinnamic ester from some other source is not inconceivable. For the simple detection of the acid, the solution obtained in the usual course of analysis, by saponifying and titrating 1.5 gms. of cinnamein is evaporated to remove alcohol, made up to 25 cc. and filtered. The filtrate (containing about 1% cinnamic acid) should give a heavy precipitate with 2 cc. of a strong solution of manganese sulphate.

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