

A STUDY OF THE CONSTITUENTS OF SIAM BENZOIN IN RELATION TO THEIR PRESERVATIVE ACTION ON LARD.\*<sup>1</sup>BY WILLIAM J. HUSA<sup>2</sup> AND DONALD E. RILEY.<sup>3</sup>

## INTRODUCTION.

The preservative action of benzoïn on lard was discovered in France by Deschamps (1) in 1843, and since that time benzoïnated lard has become official in a dozen of the leading pharmacopœias of the world (2). Although Siam benzoïn is widely used as a preservative of fats, the mechanism of its effect of retarding the development of rancidity has not been well understood. The stabilizing effect of benzoïn was commonly ascribed to the antiseptic action of its constituents, until Husa and Husa (3), in 1926, called attention to the inadequacy of this explanation and indicated that the benzoïn probably functions as a negative catalyst of oxidation. A search of the literature having shown a lack of agreement as to which constituents of benzoïn are effective in retarding the rancidity of lard, the effect having been variously ascribed to benzoïc acid, cinnamic acid, volatile oil, resin and odorous constituents, Husa and Husa (3) carried out experiments which proved that benzoïc acid and cinnamic acid are not effective in retarding the development of rancidity in lard. Although the value of benzoïn as a preservative of lard seemed well established in the older literature, some question arose as to whether or not it really exerted a preservative action, hence some experiments were carried out on this point in 1930 by Husa (4) and it was found that plain lard deteriorated several times as rapidly as benzoïnated lard.

It having thus been shown that Siam benzoïn has a definite preservative action on lard, due to some constituents other than benzoïc and cinnamic acids, the present investigation was carried out with the purpose of determining which constituents of Siam benzoïn are responsible for the retardation of rancidity.

## HISTORICAL REVIEW.

*Constituents of Siam Benzoïn.*—Benzoïc acid was obtained by the destructive distillation of benzoïn as early as 1556 (5). The acids of benzoïn were studied by Kolbe and Lautemann (6) and by Lowe (7); the latter showed that at least some of the benzoïc acid was present in the free state. Curtiss (8) obtained a higher yield of benzoïc acid by sublimation than by extraction. Schlickum (9) attempted to separate the constituents of benzoïn and Moody (10) reported the presence of cinnamic acid in benzoïn.

In 1893, Fritz Ludy, under the direction of A. Tschirch, made the first thorough investigation of the constituents of Siam benzoïn (11). Ludy found that Siam benzoïn consisted mainly of a mixture of benzoïc acid esters of two alcohols, benzoïresinol and siaresinotannol. Benzoïresinol,  $C_{18}H_{26}O_2$ , was a white crystalline substance, melting at 272–274° C.; several derivatives were prepared. Siaresinotannol,  $C_{12}H_{14}O_3$ , was a brown, amorphous substance. Ludy isolated the mixture of the two esters by repeated precipitation of an ethereal solution with petroleum ether, re-

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moving the last traces of free benzoic acid with 0.1% NaOH solution. This produced a light yellow, resin-like, transparent, brittle, odorless and tasteless mass melting at 65° C. Upon saponification with KOH, it yielded 38.2% benzoic acid, 56.7% siaresinotannol and 5.1% benzoeresinol. Besides this resin mixture, Ludy found that Siam benzoin contained 0.15% vanillin, some free benzoic acid and 0.3% of an oily, aromatic, neutral liquid which proved to be an ester of benzoic acid; the alcohol of the ester was thought to be possibly cinnamyl or benzyl alcohol, but owing to the small quantity was not definitely identified.

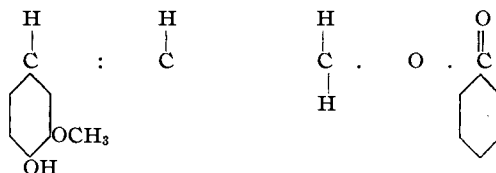
In the same year, an article by J. Salkind (12) contained statements that in Siam benzoin the benzoic acid is present as the ester of benzoeresinol and resinotannol, with a small quantity free, that vanillin was present and that cinnamic acid and benzyl benzoate could not be detected.

Cocking and Kettle (13) reported on the analytical characteristics of benzoin.

Our present knowledge of the constituents of Siam benzoin is based largely on the investigations carried out by Friedrich Reinitzer (14). He stated that Siam benzoin in the form of tears is entirely crystalline, melting at 59° C., and that upon warming to 40–50° C., its color changes to yellowish red and it finally becomes brown and amorphous due to oxidation.

Reinitzer concluded that in its pure state, Siam benzoin consists mainly of crystalline coniferyl benzoate mixed with some free siaresinolic acid and benzoic acid and a small proportion of cinnamyl benzoate. On standing in presence of air, the coniferyl benzoate tends to change to the brown amorphous form, probably with some oxidation and polymerization, the change being hastened by heat and light. Traces of vanillin appear in older samples of benzoin.

Coniferyl benzoate,  $C_{17}H_{16}O_4$ , was obtained in colorless, monoclinic crystals, m. p. 72.8° C., best preserved in the dark at a low temperature in absence of air, to prevent change into the brown amorphous form. This compound was called lubanol benzoate at first, but later lubanol was found to be identical with coniferyl alcohol. According to Zinke and Dzrimal (15) coniferyl alcohol is identical with guaiacresinol, laricresinol and pinoresinol, and the structure of coniferyl benzoate is as follows:



Coniferyl benzoate.

Siaresinolic acid was obtained in crystals, m. p. 279° C.; it was not affected by oxygen and did not turn brown. Zinke and Lieb (16) reported a m. p. of 280–281.5° C. for siaresinolic acid, and found the formula to be  $C_{30}H_{48}O_2 \cdot COOH$ .

The composition of Siam benzoin was reported by Reinitzer as follows:

Constituent.	Fresh Resin.	Old Resin.
Crystalline coniferyl benzoate	77.8%	67.8%
Amorphous coniferyl benzoate	..	10.0
Free benzoic acid	11.7	11.7
Siaresinolic acid	6.0	6.0
Cinnamyl benzoate	2.3	..
Cinnamyl benzoate and vanillin	..	0.3
Water	2.2	2.2
"Geweberste"	..	2.0
	100.0%	100.0%

The amorphous coniferyl benzoate is present in varying amounts, depending on the age and conditions of storage of the resin.

*Tests for Rancidity.*—The first step in the development of rancidity is the addition of a molecule of oxygen at the double bond of an unsaturated fatty acid, forming a peroxide. In the second

step, the fat is oxidized by the peroxide, forming aldehydes, ketones and other compounds responsible for the characteristic odor and taste of rancid fats (17). Some of the tests for rancidity are based on detection or determination of the peroxides formed in the first step, while others involve the aldehydes, ketones, etc., formed in the second step.

#### EXPERIMENTAL PART.

*Method of Determining Rancidity.*—It has long been known that rancid fats liberate iodine from potassium iodide. Since the amount of iodine liberated from KI by a rancid fat gives a measure of the peroxides present in the fat, this principle has been used as the basis of a quantitative determination of incipient rancidity. A number of methods are available based on this principle, differing only in details. Four such methods were tried, including those of Szahlender (18), Wheeler (19), Caldwell and Dye (20) and Taffel and Revis (21); in our hands Wheeler's method appeared to give somewhat more consistent results than the others and hence it was used in the experiments which follow. Wheeler's method is as follows:

From 3 to 10 Gm. of oil is dissolved in 50 cc. of a mixture of 60% glacial acetic acid and 40% chloroform and 1 cc. of saturated solution of KI is added. The mixture is stirred by a rotary motion of the flask and after 1 minute 100 cc. of water is added and the liberated iodine titrated with 0.1*N* or 0.01*N* sodium thiosulphate, using starch as indicator.

In the data which follow, rancidity is reported in terms of the number of cc. of 0.01*N* sodium thiosulphate solution required to decolorize the iodine liberated from KI by 1 Gm. of the fat; this value has been designated as the "degree of rancidity" by Szahlender (18) and this expression is used in the present report.

A substance might interfere with the test used by liberating iodine, preventing the liberation of iodine or absorbing it after it was set free. This possibility was eliminated, each substance being tested by adding it to lard of known degree of rancidity and analyzing the mixture as usual.

*Effect of Varying Percentages of Benzoin.*—Deschamps originally used 40 Gm. of benzoin per Kg. of lard. The B. P., the British Pharmaceutical Codex and the French Codex specify 30 Gm. of benzoin per Kg., while the U. S. P. amount is 10 Gm. per Kg. Hence experiments were conducted, using varying proportions of benzoin, incorporated by the U. S. P. method, with the exception that closed vessels were used.

TABLE I.—EFFECT OF VARYING PERCENTAGES OF BENZOIN ON THE RATE OF DEVELOPMENT OF RANCIDITY OF LARD. SAMPLES STORED IN AN OVEN AT 50° C.

Percentage of Benzoin.	Degree of Rancidity after				
	0 Days.	1 Day.	4 Days.	12 Days.	27 Days.
0.00	0.18	0.53	9.46	29.40	29.98
0.25	0.18	0.31	0.67	1.15	1.82
1.00	0.17	0.26	0.47	0.95	1.13
2.00	0.17	0.20	0.42	0.84	0.98
5.00	0.17	0.19	0.41	0.76	0.82

The results in Table I illustrate the definite preservative effect of Siam benzoin and indicate that with increasing quantities of benzoin lard becomes increasingly stable toward autoxidation, although the use of 2% and 5% of benzoin does not

show much advantage over 1%, which is the U. S. P. proportion. Some experiments using 3% benzoin likewise showed that this proportion has practically no advantage over 1%. Results similar to those shown in Table I were obtained in repetitions of the experiment, the samples in some cases being stored in diffused light at room temperature. The samples were stored in colorless glass ointment jars. More rapid results were obtained in the 50° C. oven, the development of rancidity being about 10 or 15 times as rapid as at room temperature.

*Preservative Effect of Volatile and Non-Volatile Portions of Siam Benzoin.*—Tests were carried out to determine whether or not the volatile constituents of benzoin are responsible for the preservative action on lard. Powdered Siam benzoin was sprinkled on glass wool in a round bottom flask placed in an oil-bath, and purified hydrogen passed in through a tube leading to the bottom of the flask; the hydrogen after passing from this flask was bubbled through lard contained in a large test-tube, which was also placed in the bath. The purpose of the hydrogen was to facilitate the passage of vapors from the benzoin into the lard. A blank determination was conducted with all conditions the same, with the exception that no benzoin was used. The operation was carried out in several steps, using fresh lard each time. The oil-bath was first held at 60° C. for two hours, and then at 90° C., 120° C. and 150° C. for two-hour periods in each case. After the 150° C. period, the bath was allowed to cool to 60° C. and the residue in the flask used to benzoinate a portion of lard. From the results of this experiment, it appeared that the preservative constituents of the benzoin remained in the portion non-volatile at 150° C., since this was the only fraction showing any preservative effect.

The experiment was repeated, with the lard kept in a separate bath at 50° C., thus eliminating overheating of the lard and reducing the likelihood of volatile constituents passing on through the lard instead of being absorbed. The bath containing the benzoin was heated for two-hour periods at 60°, 90°, 120°, 150° and 200° C., and the residue used for benzoinating a portion of lard as before. The results verified the previous conclusion that the preservative constituents were non-volatile at 150° C., but in this case the residue remaining after 200° showed no appreciable preservative effect, nor was such an effect noticeable in the fraction volatile at 200°. It seems likely that the benzoin decomposed at 200°, as the residue was quite dark in color; in this connection Reinitzer reported that benzoin gave up benzoic acid when heated to 120–140° C., and if heated more strongly, gave odors of eugenol and finally of guaiacol.

Since the results of both tests indicated that the preservative constituents were non-volatile at 150° C., attention was next devoted to methods of isolation of the non-volatile constituents of Siam benzoin.

*Isolation of Constituents of Benzoin.*—The coniferyl benzoate was isolated by a method recommended by Reinitzer (14). Large tears of benzoin, as free as possible from the brown crust, were washed with enough ether (chilled to 5° C.) to partially cover the benzoin. The mixture was frequently shaken and carefully observed. When the tears began to appear white, the ether was poured off, this treatment serving to remove the brown crust. The residue was dissolved in ether at room temperature, filtered, and petroleum ether added until the solution became turbid. Then the flask was loosely stoppered and set aside at 0° C. to crystallize. After 12 to 20 hours, crystals were obtained, usually melting at 65° to 68° C. Upon repeated recrystallization in the same manner, the coniferyl benzoate was obtained in crystals of m. p. 72.2° C. (corr.), as compared with the m. p. of 72.8° C. reported by Reinitzer. To obtain such a product, eight to twelve recrystallizations are required. For the preliminary work, the substance was recrystallized four or five times, and a product melting at 69° to 70° C. was obtained. For the final experiments, the product of highest purity was used.

The siaresinic acid was separated and purified by one of the methods given by Reinitzer. A mixture of benzoin and 70% acetic acid was allowed to stand, with occasional shaking, until the pieces of resin had disappeared, leaving a layer of light yellow material in the bottom of the flask. After filtration, the residue was washed with 70% acetic acid and then with water; the crystalline mass was then dissolved in hot alcohol and water added until the solution showed a slight turbidity, the mixture then being set aside to crystallize. By this method, crystals melting from 263.3° to 265.5° C. (corr.) were obtained. Repeated recrystallization from alcohol gave a

product melting at 268.9° to 270.0° C. (corr.). Neither of these crystalline products showed the presence of coniferyl benzoate by use of the ferric chloride color test.

Cinnamyl benzoate was prepared by treating cinnamyl alcohol with benzoyl chloride.

*Effect of Constituents of Siam Benzoin on Rate of Development of Rancidity of Lard.*—In testing the effect of the various constituents of Siam benzoin, they were added to lard in the proportions that would be present in benzoinating lard by the U. S. P. method on the basis of the analysis of Reinitzer. Thus the amount of crystalline coniferyl benzoate used in the lard was 77.8% of the amount of Siam benzoin that would be used in the U. S. P. method, and the corresponding proportions were 6% for siaresinolic acid and 4% for cinnamyl benzoate (the latter being greater than would be present in the U. S. P. amount of benzoin). Cinnamyl benzoate was tested in the proportion of 1%, as it was thought that the sample of cinnamyl benzoate used probably contained some unchanged cinnamyl alcohol. The results are given in Tables II and III.

TABLE II.—EFFECT OF CONSTITUENTS OF BENZOIN ON THE RATE OF DEVELOPMENT OF RANCIDITY OF LARD. SAMPLES STORED IN AN OVEN AT 50° C.

Added Substance.	Degree of Rancidity after				
	1 Day.	6 Days.	14 Days.	35 Days.	70 Days.
None	0.36	2.08	6.50	13.20	14.85
Siaresinolic acid	0.34	1.97	6.02	10.93	12.55
Vanillin*	0.37	1.59	5.50	9.31	10.82
Coniferyl benzoate	0.35	0.59	0.76	1.46	1.55
Benzoin	0.38	0.64	0.80	1.44	1.51

\* Added in proportion of 0.1%.

TABLE III.—EFFECT OF VARIOUS SUBSTANCES ON THE RATE OF DEVELOPMENT OF RANCIDITY OF LARD. SAMPLES STORED IN AN OVEN AT 50° C.

Added Substance.	Degree of Rancidity after				
	4 Days.	10 Days.	21 Days.	35 Days.	42 Days.
None	0.41	1.85	2.70	8.25	14.10
Cinnamyl benzoate	1.12	1.91	2.80	6.32	13.02
Cinnamyl alcohol	1.91	2.02	2.24	6.05	12.97
Benzoic acid*	0.45	1.55	2.52	7.77	13.85
Benzoin	0.26	0.61	0.77	0.83	0.96

\* 0.1 Gm. used in 50 Gm. lard.

The results indicate that coniferyl benzoate is the ingredient of Siam benzoin responsible for the preservative action on lard. Siaresinolic acid has practically no preservative effect. Vanillin gives some protection when present to the extent of 0.1%, but this proportion is higher than could be obtained from the U. S. P. amount of Siam benzoin. Cinnamyl benzoate showed no effect when used in the quantity that would be present in the U. S. P. amount of Siam benzoin and further tests showed that it was likewise ineffective when more than 25 times this proportion was used. Cinnamyl alcohol had no effect. The results with benzoic acid verified the previous report of Husa and Husa (3) that this compound has no effect on the development of rancidity in lard.

It had previously been found by Husa and Husa (3) that Siam benzoin gives a pronounced red color in the Kreis test for rancidity. In the present study, it was

found that coniferyl benzoate and vanillin similarly interfered with the use of the Kreis test. However, since siaresinolic acid gave no color with the Kreis reagent, the Kreis test was used in following the rancidity of lard alone and lard containing siaresinolic acid. The results fully confirmed the lack of preservative effect on the part of siaresinolic acid.

As a further check on the results, observations were made as to the color changes in samples of ointment of potassium iodide, using the methods described by Husa (4). The results verified the previous conclusions as to the effects of coniferyl benzoate, siaresinolic acid and vanillin.

The results with coniferyl benzoate, siaresinolic acid, benzoic acid and benzoin were also confirmed by the von Fellenberg (22) test for rancidity, this being a color test based on reaction of a fuchsin-sulphurous acid reagent with the aldehydes of the rancid fat.

Following the regular procedure using Wheeler's test, it was found that Siam benzoin which had been stored for three months in an oven at 50° C., during which time it had turned to a reddish brown color, showed very inferior preservative power as compared with U. S. P. benzoin.

Since Siam benzoin does not dissolve completely in lard in the U. S. P. benzoination process, it was of interest to observe the solubility in lard of the various constituents of benzoin. In the proportions designated in the experiments, coniferyl benzoate and vanillin dissolved completely, while siaresinolic acid dissolved only partially.

It has generally been found that negative catalysts of oxidation are themselves susceptible to oxidation; the lack of stability of coniferyl benzoate in air is thus in accord with the general rule.

#### CONCLUSIONS.

The constituent of Siam benzoin responsible for the preservative effect on lard is coniferyl benzoate. Vanillin gives some retardation of the development of rancidity when present to the extent of 0.1%, but this proportion is higher than could be obtained from the U. S. P. proportion of benzoin. The other constituents of Siam benzoin, *i. e.*, siaresinolic acid, benzoic acid and cinnamyl benzoate, do not exert a preservative effect on lard.

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## A COMPARATIVE STUDY OF MARYLAND SENNAS.\*<sup>1</sup>

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### I. INTRODUCTION.

The Maryland sennas, with the exception of *Cassia Medsgeri*, were studied by botanists as far back as the seventeenth century. At that time, Tournefort (1656-1708) placed them in the tribe *Cassia* and later Linné, in his *Species Plantarum* left this classification unchanged. Willdenow, in his work, also accepted the classification of his predecessors. However, in recent years botanists have questioned this classification and have divided the Maryland sennas into two Groups—*Cassia*, which includes *Cassia Marilandica* and *Cassia Medsgeri*; and *Chamæcrista*, which includes *Cassia nictitans* and *Cassia Chamæcrista*. As a result, various papers have been written for and against the separation of the Maryland sennas but up to the present time, no definite conclusions have been reached.

Most of the work attempting to decide the question has been carried out only upon the flowers and pods. In this paper the leaflets of the Maryland sennas are studied and from their study it appears that results are obtained which will be of value in deciding this question. As the study progressed, it became evident that the leaflets of the Maryland sennas possessed characteristics differing greatly from those of the official senna leaflets and it was decided best to include their study with that of the Maryland sennas.

The leaflets of six sennas, therefore, were studied, the two official sennas, those of *Cassia Senna* and *C. angustifolia*, and those of the four Maryland sennas, namely, *C. Marilandica*, *C. Medsgeri*, *C. nictitans* and *C. Chamæcrista*. The official senna leaflets were obtained from stock while the Maryland sennas were obtained in the vicinity of Baltimore, Md.; *C. Marilandica* being found at Owings Mills, Md., and

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<sup>1</sup> From the laboratory of Charles C. Plitt, Professor of Botany and Pharmacognosy, School of Pharmacy, University of Maryland. Thesis submitted to the Faculty of the Graduate School of the University of Maryland in partial fulfillment of the requirements for the degree of Master of Science.