

Impurities. These black acids were distilled without Twitchell-splitting in a glass laboratory batch still at 6- to 12-mm. pressure. For comparison, untreated samples from raw cottonseed soda ash foots were acidulated, Twitchell-split, and distilled; the data for these tests are given in Table VIII. It is seen that the yield of distillate from a treated still charge was about 86-89% for both expeller and hydraulic stock; the latter stock in an untreated charge gave about 69-70% yield. Complete removal of Insoluble Impurities by centrifuging might increase by another 1% the yields from treated stock. In all cases the distillation was stopped when the distillate showed an appreciable yellow color, generally about 250-260°C. Insufficient data are available yet to relate still yields to the impurities ratio of the charge, but the results of Table VIII indicate roughly an inverse proportionality.

Summary

The nature of the impurities in vegetable oil foots renders them resistant to treatment by acids as in normal acidulation. Strong caustic was found to attack the gums and make them at least partially soluble in a 5 to 10% aqueous caustic solution. At this concentration a soap phase of 45-60% TFA could be centrifugally separated, and the majority of the impurities or their degraded products were discharged in the lye. The ratio of Oxidized Acids and Insoluble Impurities to the TFA was much lower in the soap

than in the raw soapstock; the Oxidized Acids/Black Acids ratio could generally be reduced to 5% or less. This reduction in impurities was found to improve laboratory still yields markedly without the necessity of Twitchell- or pressure- splitting the black acids from treated stocks. Distillate yields of 86-89% were obtained from treated stocks, compared to 69-70% from untreated stocks.

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Pyrethrum Synergists in Sesame Oil. Sesamol, a Potent Synergist

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SESAME oil, obtained from seeds of the plant *Sesamum indicum* (L.), was found, to the exclusion of all other oils tested, to increase the insecticidal potency of pyrethrins (12). Haller and his co-workers (14, 15) fractionated sesame oil by molecular distillation and, from the two most active fractions, isolated sesamin, a compound exhibiting marked synergism with pyrethrins. By a systematic examination of compounds similar to sesamin, these workers made the very important discovery that the intact methylenedioxyphenyl group is essential for sesamin's synergistic activity (16). That optical activity had no appreciable effect on the synergistic activity of sesamin was illustrated by the fact that asarinin and isosamin, optical isomers of sesamin, were equally effective pyrethrum synergists. As predicted by them, their discovery led to the development of synthetic synergists of commercial importance.

Haller and co-workers recognized that considerable synergistic activity remained after the removal of sesamin, as did subsequent workers (18). However no other synergists in sesame oil have been thus far identified. The present research was undertaken in an effort to identify in the oil any compound or compounds other than sesamin that exert any appreciable synergism with pyrethrins so that the synergistic action of sesame oil might be fully accounted for.

Experimental

To accomplish this purpose it was decided to fractionate the oil by means of chromatography. Since the work of Haller and co-workers indicated that the strongly ultraviolet-absorbing methylenedioxyphenyl group was essential for activity as a pyrethrum synergist, it was believed that it would be possible to detect and roughly estimate the presence of any synergist by following the ultraviolet absorbance of the eluate fractions. This proved to be true. Furthermore the technique employing ultraviolet absorbance ratios previously used in the separation of alkaloids could be used to isolate and identify pure compounds from zones that separated incompletely (3).

Chromatography of Crude Sesame Oil. Partition and adsorptive chromatography were tried. The best separations were obtained by the latter technique on silicic acid.

A chromatographic column 5.5 cm. inside diameter and 31 cm. long, fitted with a sintered-glass disk, was used. A slurry of 300 g. of silicic acid (Merck) in isooctane (Phillips pure grade redistilled) was introduced into the column in three portions. The column was prewashed with 400 ml. of 12% ethyl acetate (N. F. anhydrous redistilled) in isooctane followed by 200 ml. of isooctane. Forty grams of crude sesame oil dissolved in 150 ml. of isooctane was added to the

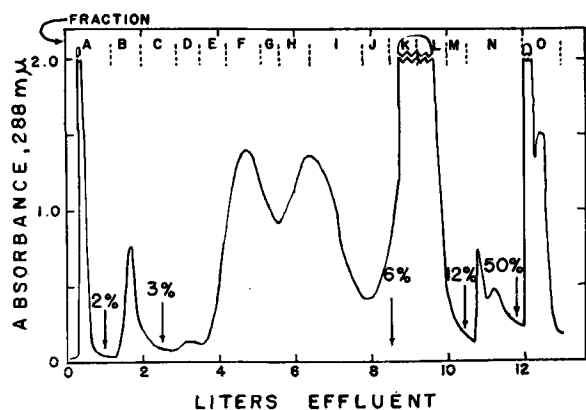


FIG. 1. Chromatography of crude sesame oil. Percentages indicate concentration of ethyl acetate in isooctane by volume used as eluant.

top of the column and washed into the gel with several small portions of isooctane. The column was then developed with isooctane followed by increasing concentrations of ethyl acetate in isooctane, as indicated in Figure 1. The absorbance of the eluate was periodically determined with a Beckman Model DU quartz spectrophotometer at 280, 288, and 300 μ to approximate the spectrum of each fraction. The elution pattern is plotted in Figure 1. The eluate was divided into 15 fractions, each of which was evaporated to dryness and weighed. From certain fractions crystals could be obtained; there were separated. Each of the resulting materials was tested for activity as a pyrethrum synergist against the house fly (*Musca domestica* L.) by the turntable method. Sesamin and sesamol were tested for synergism at a concentration of 1 mg./ml., and other chromatographic fractions at 8-10 mg./ml., in 1:9 acetone-Deobase. The data obtained from this study are summarized in Table I.

TABLE I
Chromatographic Fractions of Crude Sesame Oil and Their Synergistic Properties with Pyrethrum^a

Fraction	Crystalline	Noncrystalline
	grams	grams
A.....	26.592
B.....	8.388
C.....	0.5258
D.....	0.0821
E.....	0.0228	0.2098
F.....	0.0159	0.2372
G.....	0.1419 ^b
H.....	0.0228 ^d	0.2517 ^b
I.....	0.0511 ^d	0.4185 ^b
J.....	0.0098 ^c	0.1632 ^b
K.....	0.1742 ^c	0.6482 ^b
L.....	0.1791 ^c	0.4965
M.....	0.1018
N.....	0.4983
O.....	0.6807

^a Fractions not marked with a footnote exhibited little or no synergism.

^b Synergistic.

^c Strongly synergistic.

^d Very strongly synergistic.

Crystalline Materials. From fractions E and F 38.7 mg. of a compound melting at 134.6° was crystallized from the cold acetone solution. In admixture with phytosterol (m.p. 137°), isolated from sesame oil as described by Budowski (8), there was no depression. Neither the phytosterol nor its mother liquor was active as a synergist.

From fractions H and I 73.9 mg. of a compound melting at 90-92° was isolated from isooctane solution. This compound could be purified by further

crystallizations from chloroform-isooctane solution so that it melted at 94°. It was identified as sesamol by melting point, ultraviolet spectrum (7), Villavecchia reaction (2), elementary analysis, optical rotation, and molecular weight.

Anal. Calcd. for C₂₀H₁₈O₇: C, 64.86; H, 4.90; mol. wt., 370. Found: C, 65.15; H, 5.06; mol. wt. (11) 379.

Sesamol is soluble in chloroform, acetone, benzene, ethyl acetate, and glacial acetic acid; less soluble in ethanol, methanol, and ether; and practically insoluble in isooctane, petroleum ether, dilute alkali, and water.

From fraction J 9.8 mg. of an impure sesamin was obtained, m.p. 115-118°.

From the isooctane solutions of fractions K and L 353.3 mg. of sesamin was obtained, m.p. 119.5-121°. A mixed-melting-point determination with an authentic sample of sesamin gave no depression. The sample was further identified as sesamin by ultraviolet spectrum (7) and optical rotation.

Noncrystalline Materials. The only noncrystalline materials that exhibited synergistic action were the mother liquors associated with the sesamol zone. Some sesamol tailed into fraction K (a sesamin fraction). In other experiments in which sesamin and sesamol were completely separated, the sesamin mother liquor had practically no activity. The sesamol mother liquors accounted for only a small portion of the total activity. They gave a positive Villavecchia test (2) for sesamol, indicating that the activity was due to unprecipitated sesamol. About 90% of the sesamol was precipitated, as determined by the quantitative method of Suarez *et al.* (21).

The total of crystalline and noncrystalline material recovered in the chromatography was 39.9 g., or 99.8%.

Ultraviolet Absorption Data. The ultraviolet absorption spectra of sesamol and sesamin in isooctane (7) are similar to those obtained from these compounds in ethyl acetate-isooctane solutions, the solvent used in the present chromatography. The sesamol and sesamin zones are easily distinguished by their absorbance ratios at 288:300 μ (about 2.1 and 7.0:1, respectively). The material preceding sesamol has a 280:288 μ absorbance ratio of about 5:1 as compared with about 0.9:1 for sesamol. The appearance of sesamol in the eluate is therefore readily recognized by the drop in this ratio. Sesamol comes off with the sesamol, and, when present in appreciable amount, it drops the 288:300 μ absorbance ratio of sesamol lower than 2:1.

While the foregoing chromatography gives data on one oil, the ultraviolet elution pattern is not typical of all sesame oils. In general, most of the crude oils expressed by the author directly from the seed had little ultraviolet absorption in the 255-320 μ region other than that due to sesamol, sesamol, and sesamin.

The above procedure may be used to isolate sesamol from commercial sesamin concentrates (which contain sesamol) as well as from sesame oil. The concentrate is dissolved in a 1:1 chloroform-isooctane solution to introduce it on the column.

Discussion

The systematic entomological examination of the chromatographic fractions of the oil showed only two

zones containing appreciable activity as pyrethrum synergists. From one of these zones sesamin was isolated. Its mother liquor had little or no activity. From the other zone sesamol was obtained. Its mother liquor was active, but this activity was due to unprecipitated sesamol. Sesamin and sesamol are therefore the only synergistic materials exhibiting appreciable activity in sesame oil.

Entomological tests have indicated that sesamol is the most powerful pyrethrum synergist thus far isolated, being about five times as effective as sesamin. Sesamol without pyrethrins, like sesamin, did not show any insecticidal action.

Recently methods for the determination of sesamol and sesamin in sesame oil (6, 7) and a modification of these methods have been published (21). These methods indicate that crude sesame oil usually contains 0.3-0.5% of sesamol and 0.5-1.0% of sesamin. On the basis of these figures and the knowledge that sesamol is about five times as effective as sesamin, it appears that most of the synergistic action of sesame oil with pyrethrins is due to sesamol rather than sesamin.

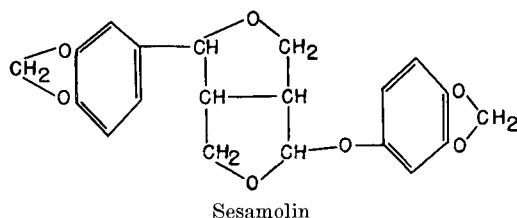
In the course of this work sesamol was tested as a pyrethrum synergist but was found to be inactive.

Chemical Studies on Sesamol

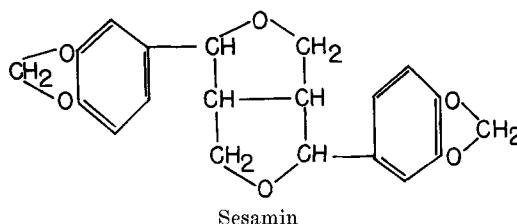
Although sesamol was originally isolated in 1903 by Canzoneri and Perciabosco (10), it was named in 1928 by Adriani (1), who assigned to it the formula $C_{20}H_{18}O_7$, m.p. 94° . The compound is hydrolyzed by dilute mineral acids, giving sesamol, m.p. 65.5° [3,4-methylenedioxyphenol] (17), and samin, $C_{13}H_{14}O_5$ (1), a compound of unknown chemical structure.

In view of the marked synergistic activity of sesamol, some studies on its chemistry were made.

From the outset of this investigation it was noted that sesamol is strikingly similar to sesamin, and, on the basis of the following data and discussion, the following tentative formula for sesamol is being advanced as a basis for future work.



Sesamin is known to have the following formula:



Sesamol contains no aliphatic double bonds since it does not decolorize potassium permanganate in acetone even when heated to 60° for several minutes. When treated with hydroxylamine hydrochloride, no acid is liberated even after boiling (20). The compound therefore contains no carbonyl groups. Sesamol

is not dissolved by alkali, even when warmed to 70° for one-half hour. This behavior indicates an absence of lactone groups. Its stability toward alkali shows that it does not contain ester groups. Bromine in carbon tetrachloride is slowly decolorized by sesamol; hydrogen bromide is evolved, indicating that the reaction is substitution rather than addition. An active hydrogen determination was negative indicating that no hydroxyl groups are present. Methoxyl and C-methyl determinations are also negative.

The ultraviolet absorption spectra of sesamin and sesamol are almost identical when compared on a molar basis. This fact suggests the close relationship between the two compounds, particularly in regard to the chromophoric groups. Sesamin's chromophores are two methylenedioxyphenyl groups. Sesamol is known to contain at least one such group because it splits off sesamol in the presence of mineral acids. The molar absorptivities (E) of the corresponding maxima of both compounds agree within less than 2% (7): sesamin, $E_{287} = 8198$, $E_{236} = 9260$; sesamol, $E_{288.5} = 8062$, $E_{235} = 9195$. One may therefore surmise that sesamol, like sesamin, possesses two methylenedioxyphenyl groups.

To verify that sesamol has two methylenedioxy groups, a modification (4) of the method of Bricker and Johnson (5) was used. By this method each mole of methylenedioxy group yields from 0.75-1.08 moles formaldehyde. Sesamin gave 2.50 and sesamol 2.54 moles of formaldehyde. This high result for sesamin was inexplicable until it was discovered that pinosresinol acetate yielded 0.79 mole of formaldehyde. Pinosresinol acetate differs from sesamin in having an acetoxy and a methoxy group in place of each of the two methylenedioxy groups in sesamin. Acetoxy and methoxy groups do not yield formaldehyde. The formaldehyde obtained from the pinosresinol acetate must therefore originate from the central nucleus which is identical with that of sesamin. The yield of formaldehyde from sesamin must therefore be explained on the basis of 0.79 mole originating from the nucleus and 1.71 moles from the two methylenedioxy groups. Likewise it is tempting to explain the yield of formaldehyde from sesamol by the presence of two methylenedioxy groups and a central nucleus identical with that of sesamin.

Further evidence falls in line with this hypothesis. Sesamol must contain one methylenedioxyphenyl and one methylenedioxyphenyl ether group attached to a central nucleus, which by difference has the formula $C_6H_8O_2$. This formula, which indicates that the nucleus is bicyclic, is identical with the empirical formula of sesamin's nucleus.

From the aforementioned tests on the whole compound it may be concluded that the two oxygen atoms in the sesamol nucleus are indifferent and are therefore present as ethers. The acetal is a form of ether that is readily split by mineral acids. The fact that sesamol is split in this manner from the central nucleus may be taken as evidence that the methylenedioxyphenyl ether group is part of an acetal linkage. Note that the carbon with the double ether linkage in the proposed formula forms an acetal.

Hydrogenolysis of sesamin in acetic acid over palladium charcoal results in the rapid uptake of two moles of hydrogen. The compound then absorbs hydrogen very slowly. The rapid uptake of the two moles of hydrogen is due to the hydrogenolysis of two

benzyl ether groups which are known to cleave readily (13). Under identical conditions sesamol takes up one mole of hydrogen rapidly and then gradually absorbs hydrogen with no break in rate. On the basis of the proposed formula sesamol would be expected to take up only one mole rapidly due to the presence of only one benzyl ether group. Hydrogenolysis data is therefore consistent with the proposed formula.

It was interesting to check whether sesamol was liberated from sesamol by hydrogenolysis. Sesamol was therefore hydrogenated as above for 22 hours, the catalyst was filtered off, and the acetic acid was evaporated. The residue (containing sesamol acetate) was saponified with alcoholic potassium hydroxide, cooled, and extracted with ether. This extract was discarded (sesamol in alkaline layer). The alkali was then neutralized and again extracted with ether. The presence of sesamol in the ether residue of the neutral extract was verified by a strong positive test for a phenol and a positive Villavecchia test. Sesamol is therefore cleaved by hydrogenolysis as well as by mineral acids. This is in agreement with Budowski's findings that hydrogenation of sesame oil (containing sesamol) produces free sesamol (9), an antioxidant which stabilizes the oil.

The remarkable similarity between sesamol and sesamin is again evident from their infrared absorption spectra (Figure 2). From 2-8 microns there is

sesamin and sesamol are found in the same source and are both pyrethrum synergists imply similarity of structure.

While the above chemical data and discussion do not prove the proposed formula for sesamol, they do shed light on the structure of a compound that may well have commercial possibilities in the future. This is especially true since the cultivation of sesame in this country is now considered a likely possibility.

It is also interesting to note that since sesamol is so much more potent a pyrethrum synergist than sesamin, more potent synergists might be forthcoming if compounds more closely related to sesamol than to sesamin are synthesized. The preparation of 3,4-methylenedioxyphenyl ether (or sesamol) derivatives has therefore been undertaken.

Summary

By the systematic entomological examination of chromatographic fractions of sesame oil two pyrethrum synergists, sesamin and sesamol, were found to account for practically all the synergistic activity of the oil. Sesamol, which had not been known to be synergistic, is about five times as active as sesamin and, even though usually present in smaller amount than sesamin, it is believed to account for most of the synergistic activity in sesame oil.

Because of sesamol's marked synergistic activity, studies on its chemistry were made, and some of its chemical properties are reported. The studies have indicated a close similarity between sesamin and sesamol, and a tentative chemical formula for sesamol has been proposed as a basis for future work.

Infrared absorption spectra of sesamin and sesamol are reported.

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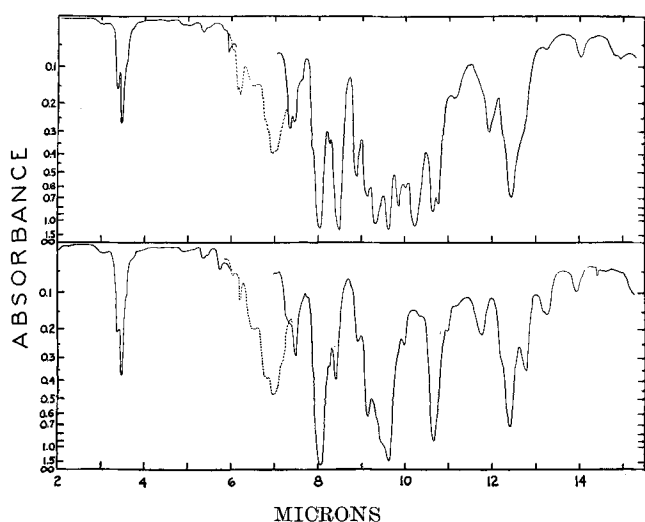


FIG. 2. Infrared spectra of sesamol (upper) and sesamin (lower) in carbon disulfide (solid line) and in chloroform (dotted line), 10 mg./ml.

practically no difference in their spectra. These data also confirm that no hydroxyl or carbonyl groups are present in sesamol. Sesamol in the proposed formula has the same ether linkages as sesamin but has the additional methylenedioxyphenyl ether linkage. It is interesting to note that in the 8-9 micron region where ethers absorb (19), both compounds have a peak at 8.05 microns and sesamol has the additional peak at 8.50 microns. Differences in the 8-11 micron region may be a fruitful source of additional evidence of structure by comparison with compounds having similar ether linkages.

Sesamin and sesamol are alike in that they have similar solubilities in various solvents. The facts that