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## *Part I*

# SUCROSE DERIVATIVES OF TALLOW:

## NEW, EFFECTIVE SURFACTANTS FOR HERBICIDE SPRAYS[a]

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Recently a new group of chemicals with some unique properties has become available in commercial quantities — sucrose esters of fatty acids (11, 18). Sucrose is inexpensive and readily available; fatty acids are obtained from tallow which is also of low cost and obtainable in large quantities as an agricultural surplus material. Indeed, it was the abundance of tallow produced by the meat packing and rendering industries which prompted us to investigate new uses of tallow and its derivatives, including sugar esters. We hoped to find an application which would satisfy a need and require large amounts of tallow. Sugar esters of fatty acids have a wide and unusual range of physical and biological properties which suggested to us their application in agricultural sprays.

The work reported here includes greenhouse and field studies on the feasibility of using sugar esters as adjuvants in herbicide sprays. The results justify the use

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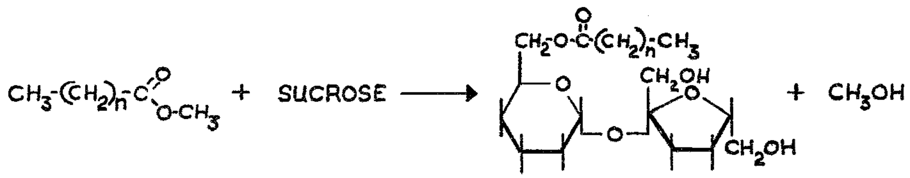
of these compounds in herbicide sprays. Indeed, sucrose derivatives of tallow are as good or better than the surfactants now used. Whether these chemicals may also be effective in pesticide and nutritional sprays remains to be found. The physical chemical, and biological properties of sugar esters make them ideally suited for agricultural use.

# The Nature of Sucrose Esters of Fatty Acids

Sucrose monoesters of fatty acids are readily prepared by alcoholysis of the methyl esters of fatty acids with sucrose (13,14). With a 3 to 1 molar ratio of sucrose to methyl ester of fatty acid and 0.1 mole of an alkaline catalyst ($K_2$ $CO_3$ ), we obtained up to 90 percent conversion of the methyl ester to the monoester of sucrose (9).




**methyl ester** **sugar** **sucrose monoester** **methyl alcohol**

A comparison of the molar proportion of the fatty acid to sucrose showed the recrystallized products to be more than 97 percent monoesters. York **et al.** (19) found that in sucrose monolaurate the fatty acid is joined primarily at the 6 position of the glucose portion of sucrose, although some fatty acid is also attached to the fructose portion of the molecule. The structures of di- and tri- esters are not known and probably vary with the method and conditions of synthesis.

Sugar esters of fatty acids may be prepared commercially by alcoholysis of methyl esters of fatty acids. If the mixture of methyl esters contains fatty acids in the same proportion as found in tallow, this method yields a product called sucrose tallowate. The sucrose esters may also be prepared in a synergic mixture by transesterification between tallow triglycerides and sucrose. This method yields a complex mixture mainly of mono- and di-esters of glucose, fructose, and sucrose, mono- and di-glycerides, free sucrose and free fatty acids. Such a mixture is referred to as a sucroglyceride. A semi-quantitative analysis of sucrose tallowates, a sucroglyceride, and sugar esters, in general, is described in a second paper (2). Other sugars and carbohydrates may be substituted for sucrose in the preparation of sugar esters, but they were not included in this study.

The chemical and physical properties of sucrose esters of fatty acids have been reviewed (8,11,12,13,14,15,16,17). In summary, sucrose monoesters of long-chain fatty acids such as palmitic, stearic, and oleic acid are dispersible in water, soluble in warm or hot organic solvents, miscible with fats, stable on heating, non-volatile, tasteless, and are mild detergents. From a biological viewpoint, one would expect sucrose esters of naturally-occurring fatty acids to be non-toxic to plants and animals; both the sugar and the fatty acids are constituents of the diet of most animals and are normally found in the cells of plants and animals. Extensive testing has not been done, but studies thus far have shown sucrose monostearate to be non-toxic to rats (12), the sugar portion of sucrose monolaurate is inverted by yeast invertase (19), a solution of 10 percent sucrose monostearate does not irritate the eyes (17), and sucrose esters may be fed to dogs at high levels without deleterious effects (1). As will be shown, we have observed no toxic effects of a wide variety of sugar esters when sprayed on bean and cotton plants.

# Greenhouse Studies

Sucrose esters were evaluated using the bean curvature test (10). The effect of each ester on enhancing the growth effect of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) was compared with one of the best surfactants available. Vatsol K.[C,D] The herbicide 2,4-D was used because of its broad use in agriculture. Vatsol K is a wetting agent which has had extensive use in dust and liquid pesticides (20).

In the bean curvature test the surfactant to be evaluated is dissolved in water with a standard amount of herbicide. Then a drop of the mixture is placed on the upper surface of one of the first foliar leaves of the bean plant. After 2 hours, if the herbicide has penetrated the leaf and has translocated to the main stem, the stem grows more rapidly on the side adjacent to the leaf to which the herbicide was applied. The amount of curvature of the stem away from its original vertical position is measured in degrees. If the amount of curvature of the test plant treated with herbicide plus surfactant is greater than the curvature of a plant to which herbicide only was applied, one may conclude that the surfactant increased the effectiveness of the herbicide. Of course, variation among plants requires the use of replicate treatments and statistical analysis of the data. Phytotoxicity of the surfactant is also determined by applying surfactant only to test plants.

Sucrose esters ($>$ 97 percent monoesters) were synthesized (9) from the methyl esters of lauric ($C_{12}$), myristic ($C_{14}$), palmitic ($C_{16}$), stearic ($C_{18}$), oleic ($C_{18}$, one double bond), linoleic ($C_{18}$, two double bonds), and linolenic acid ($C_{18}$, three double bonds). This range of esters allowed for evaluation of the fatty acids (stearic and oleic) which are the major components of tallow and determination of the influence of chain length and degree of unsaturation in promoting the movement of the herbicide in the plant.

Bean plants *Phaseolus vulgaris* L., Variety Ferry-Morse Pole Number 191 were selected for uniformity on the basis of size, length of the second internode, and uniform unfolding of the first foliar leaves. The plants were about 21 days old. There was only one plant per pot and the plants received sunlight and artificial illumination from above from cool daylight fluorescent lights so the stems were straight and erect. All plants were watered 3 hours before treatment. Other details of the experimental procedure are described elsewhere (9). The concentration of 2,4-D used was determined by preliminary studies which showed that 0.01 ml of

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[C] Vatsol K is a dry powder containing 33 percent sodium dioctylsulfosuccinate, and is a product of the American Cyanmid Company.

[D] The mention of commercial products and companies anywhere in this paper does not imply that they are endorsed or recommended by the Arizona Agricultural Experiment Station over others of a similar nature not mentioned.

## *Table 1. Effect of surfactants on enhancing the growth effect of 2,4-D.*

MEAN STEM CURVATURE OF THREE BEAN PLANTS AFTER TWO HOURS.
The pH of each test solution is indicated.

| Surfactant | Mean stem curvature | | | |
|---|---|---|---|---|
| | Test 1 pH 3.4 | Test 2 pH 3.4 | Test 3 pH 4.3 | Test 4 pH 4.3 |
| | curvature in degrees | | | |
| Sucrose laurate | 21 | --- | 32 | 34 |
| Sucrose myristate | --- | 31 | 17 | 23 |
| Sucrose palmitate | --- | 27 | 11 | 21 |
| Sucrose stearate | 27 | --- | 24 | 43 |
| Sucrose oleate | --- | 11 | 19 | 17 |
| Sucrose linoleate | --- | 30 | 23 | 33 |
| Sucrose linolenate | 31 | --- | 51 | 37 |
| Vatsol K | 20 | 21 | 33 | 41 |
| None | 21 | 18 | 18 | 28 |

## *Table 2. Comparison of the effects of Vatsol K, sucrose linolenate, and sucrose stearate in enhancing the growth curvature caused by 2,4-D.*

Means underlined by the same line are not significantly different at the 5 percent level.

| Plant Number | Treatment | | | |
|---|---|---|---|---|
| | No Surfactant | Vatsol K | Sucrose Linolenate | Sucrose Stearate |
| | curvature in degrees | | | |
| 1 | 1 | 8 | 14 | 15 |
| 2 | 2 | 11 | 18 | 12 |
| 3 | 1 | 10 | 10 | 13 |
| 4 | 1 | 10 | 15 | 18 |
| 5 | 3 | 8 | 14 | 16 |
| 6 | 1 | 8 | 14 | 12 |
| 7 | 3 | 16 | 12 | 20 |
| 8 | 4 | 11 | 12 | 21 |
| 9 | 1 | 13 | 15 | 15 |
| 10 | 1 | 19 | 15 | 20 |
| Mean Curvature | 1.8 | 11.4 | 13.9 | 16.2 |

solution containing 200 ppm 2,4-D (as the free acid) at a pH of 3.4 or 4.3 produced a curvature of 20° in two hours. All solutions contained 50 percent ethyl alcohol and were buffered to the low pH as suggested by Crafts (3). The concentration of Vatsol K or sugar ester was 0.1 percent (w/v).

These tests (Table 1) showed that some of the sugar esters and Vatsol K enhance growth curvature caused by 2,4-D. Results from another curvature test (Table 2) were analyzed statistically using the new multiple range test (4,7). In this test sucrose stearate was more effective than Vatsol K and sucrose linolenate was as effective as Vatsol K in enhancing the growth effect of 2,4-D.

A series of greenhouse studies utilizing radioactive 2,4-D (2,4-dichlorophenoxyacetic acid-1-$C^{14}$) was also made to determine directly if the movement of the herbicide is enhanced (9). Autoradiographs of plants treated with the radioactive 2,4-D and surfactant showed qualitatively little or no difference between the rate of penetration and movement of 2,4-D in plants treated with sucrose esters or Vatsol K.

# Field Trials

**Methods.** The greenhouse studies showed that sucrose stearate, the sugar ester of the principal fatty acid found in tallow, enhances herbicide activity. Other sucrose esters also were shown to be effective. On the basis of these results, extensive field tests were made using small plots of cotton and commercial sugar ester products and surfactants.

The field trials were conducted in the spring and summer of 1963 and 1964. Temperatures during these periods ranged from 60° to 100° F and the relative humidity ranged from 10 to 75 percent. Materials were applied on essentially windfree days and not more than traces of rain occurred during the tests.

The amino form of 2,4-D, as recommended for Arizona's high temperatures (5), was used in order to minimize vaporization. A solution of 2,4-D amine was prepared by titrating the free acid of 2,4-D with diethylamine in an aqueous solution until all acid dissolved. The solution was diluted with water to obtain the desired concentration of 2,4-D, calculated on the weight of the free acid. All formulations were applied with a Hudson knapsack sprayer calibrated to deliver 40 gallons/acre at 40 psi and a speed of 3.4 mph. The drop in pressure during application was not more than 4 psi; this varied the amount delivered by less than 2 percent. Dispersions of surfactants at all concentrations used were put through the sprayer at the designated pressure.

As the analysis by thin-layer chromatography shows (Table 3), the first three of these products are complex mixtures.

Three commercial surfactants which have been shown to enhance herbicidal activity (6) were used as references in the field tests:

**Tween 20 (polyoxyethylene, sorbitan monolaurate), non-ionic surfactant, from Atlas Chemical Industries, Inc., Chemicals Division, Wilmington 99, Delaware.**

**Dupanol WAQ (sodium lauryl sulfate), anionic surfactant, from E. I. DuPont de Nemours and Company, Organic Chemicals Dept., Dyes and Chemicals Division, Wilmington 98, Delaware.**

**Colloidal X-77 (alkylarylpolyoxyethylene glycols, free fatty acids, and isopropyl alcohol), blended surfactant, from Colloidal Products Corporation, 100 Gate 5 Road, Sausalito, California.**

Four commercial tallow products were compared in the field tests:

| Product | Supplier | Code Name |
|---|---|---|
| Sucrose Monotallowate Tech. (Lot 20, Sample 2075-1) | Colonial Sugars Co., Sucro-Chemical Div., Gramercy, Louisiana | SET-1 |
| Sucrose Monohydrogenated Tallowate (Lot 21, Sample 2076-1) | Colonial Sugars Co. | SETH-1 |
| Sucrose Tallow Glyceride (Sucroglyceride) | Ledoga S.p.A., Via Roberto Lepetit 8, Milano, Italy | T-110 |
| Polyoxyethylenated Sucroglyceride (Ledoga Detergent Base Number One) | Ledoga S.p.A. | LDB-1 |

Cotton (*Gossypium hirsutum* L.) varieties used in these studies were Acala-44-10 in 1963 and Deltapine Smooth Leaf in 1964. Cotton was planted at time intervals in the spring and summer of 1963 and 1964 so as to provide a continuous supply of plants of the maturity desired. The land was fertilized and irrigated before planting. When the soil surface was dry, the soil was harrowed and 10 rows were seeded in moist soil under a dry mulch. The rows were 36 inches apart and approximately 525 feet long. All sprays were applied when the cotton plants were 5 to 6 inches tall and in the 6 to 8-leaf stage. Spraying was done immediately after sunrise. All treatments, including controls, were replicated 5 or 6 times in a randomized complete block design. Each plot consisted of a row 25 feet long.

At a given time after treatment, usually 10 days, every third plant in each plot was pulled until 20 plants were harvested. No plants were taken from the 2.5

**Table 3. Semi-quantitative analysis by thin-layer chromatography of three commercial tallow products.**

| Constituent | Product SET-1 | Product SETH-1 | Product T-110 |
|---|---|---|---|
| | % | % | % |
| mono-esters of sucrose | 65 | 85 | 25 * |
| di-esters of sucrose | 20 | } | 12 |
| tri-esters of sucrose | } | } 15 | 12 |
| methyl esters of fatty acids | } 15 | } | --- |
| sucrose | } | | trace |
| monoglycerides | --- | --- | 30 |
| diglycerides | --- | --- | 12 |
| triglycerides | --- | --- | 8 |

* May also contain esters of glucose and fructose.

feet of row at the ends of each plot. The harvested plants from each replicated plot were placed in a paper bag and dried at 105° C in a forced draft oven. When necessary, harvested plants were stored at 4°C until space was available for drying in the oven. A second group of 20 plants was harvested from each plot several days later by pulling every second plant in the row. In a few tests, 20 plants of those remaining were harvested from each plot; these constituted a third harvest. In the few cases where the stand was so poor that there were not 60 plants in the plot, the number harvested was converted to a 20-plant basis after weighing.

Replications of each treatment in the tests of 1963 were treated as equal units in a statistically small sample and treatments within an experiment were compared using the Student "t" test (7). Because no two experiments were made under identical conditions, no statistical tests were used to compare treatments between two experiments. The tests made in 1964 were evaluated using Duncan's multiple range criteria (4). In all analyses, the 5 percent level (P is 0.05) is taken as significant, whereas the 1 percent level (P is 0.01) is taken as highly significant.

# Field Trials in 1963

The amount of 2,4-D amine (calculated as the free acid) needed to cause a significant reduction of dry weight of cotton plants 10 and 20 days after treatment was determined by spraying plants with 1/32 to 1.0 pound 2,4-D per acre (Figure 1). The rate selected for use in the 1963 experiments was 1/16 pound 2,4-D per acre.

Phytotoxicity tests of the surfactants were made by spraying 0.01, 0.1, and 1.0 percent (w/v) solutions of surfactants on cotton (Table 4). Solutions of each surfac-

***Table 4. Determination of phytotoxicity of surfactants by their effect on the dry weight of cotton plants 10 days after application.***

| Surfactant | | | |
|---|---|---|---|
| Name | Conc. | Relative difference of dry weight from the control | P[1] |
| | % | % | |
| T-110 | 0.01 | + 3 | 0.4 |
| | 0.1 | + 6 | 0.5 |
| | 1.0 | — 4 | 0.8 |
| SET-1 | 0.01 | + 10 | 0.3 |
| | 0.1 | + 13 | 0.1 |
| | 1.0 | + 3 | 0.8 |
| SETH-1 | 0.01 | + 6 | 0.4 |
| | 0.1 | + 17 | 0.1 |
| | 1.0 | + 8 | 0.8 |

[1] Probability that the treatment is the same as the control.

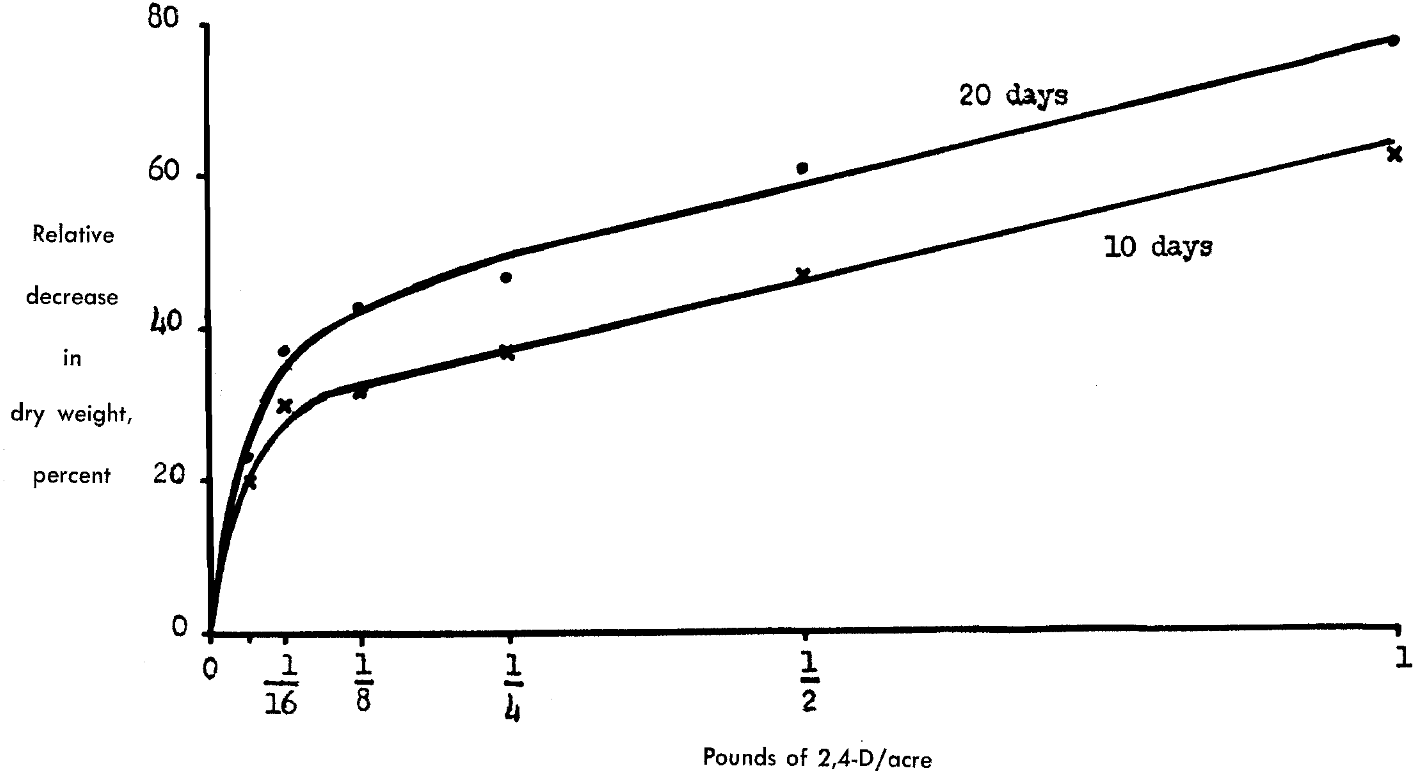


FIGURE 1. Effect of concentration of 2,4-D on reduction of dry weight of cotton plants 10 and 20 days after treatment. For the 20-day curve, the probability is less than 0.01 than any treatment was the same as the control (0 pound 2,4-D/acre).

tant were applied at the rate of 40 gallons per acre. At this rate of application, a 1.0 percent solution of surfactant is equivalent to applying 3.3 pounds of surfactant per acre. No significant differences were found between treatments and the controls. Variation of the cotton plants used in this study resulted in high standard deviations, but even where the effect of treatment approached significance, no plants showed necrosis or any visible effects of the surfactants. No mold growth occurred on the plants even though the sugar esters coated the leaf surfaces with possible substrates for mold growth.

Having established the lack of phytotoxicity and the minimum dosage of 2,4-D needed to cause a highly significant growth effect, enhancement by the surfactants of the growth response was next studied. The surfactants were tested at 0.01, 0.1, and 1.0 percent concentrations (w/v). Also one spray treatment contained water only, and a second spray contained only aqueous 2,4-D amine. Plants were harvested after 10 and 20 days (Table 5).

At a concentration of 0.01 percent, both T-110 and SETH-1 showed significant enhancement of 2,4-D activity, and T-110 was significantly more effective than Tween 20. At a concentration of 0.1 percent, all surfactants were equally effective

## *Table 5. Relative reduction (percent) of dry weight of cotton plants treated with 1/16 pound 2,4-D per acre and surfactants.*

Numbers following each mean indicate results of other treatments which are significantly different. Underlining of these numbers indicates differences which are highly significant.

| Treatment Number and Surfactant | Percent concentration of surfactant | | | | | |
|---|---|---|---|---|---|---|
| | 0.01 | | 0.1 | | 1.0 | |
| | Days after treatment | | Days after treatment | | Days after treatment | |
| | 10 | 20 | 10 | 20 | 10 | 20 |
| | relative reduction in dry weight, % | | | | | |
| 1. None and no 2,4-D | 0 | 0 | 0 | 0 | 0 | 0 |
| 2. None | 26 1 | 41 1 | 26 1 | 37 1 | 14 1 | 21 1 |
| 3. T-110 | 32 1 | 58 1,2,7 | 35 1 | 57 1,2 | 40 1,2 | 59 1,2, 6,7 |
| 4. SET-1 | 29 1 | 49 1 | 35 1 | 61 1,2 | 40 1,2 | 59 1,2, 6,7 |
| 5. SETH-1 | 29 1 | 53 1,2 | 44 1,2 | 57 1,2 | 36 1,2 | 53 1,2 |
| 6. WAQ | 32 1 | 48 1 | 35 1 | 54 1,2 | 31 1,2 | 45 1,2 |
| 7. Tween 20 | 27 1 | 47 1 | 43 1,2 | 58 1,2 | 32 1,2 | 47 1,2 |
| 8. X-77 | 26 1 | 50 1 | 38 1 | 61 1,2 | 38 1,2 | 59 1,2, 6 |

in enhancing the herbicidal activity of 2,4-D. At a concentration of 1.0 percent, all surfactants were still effective but both T-110 and SET-1 were more effective than WAQ and Tween 20.

To further test the effect of sugar-tallow surfactants in enhancing the activity of 2,4-D, the herbicide was applied at higher concentrations which would give increased kill of plants (Tables 6 and 7). The rates of 2,4-D used are those recommended for control of broadleaved plants in Arizona (5). Dry weights of plants 7 and 14 days after treatment and percent kill 21 days after treatment were measured. The surfactants were formulated at two concentrations, 0.25 and 0.5 percent. X-77, the commercial surfactant found in previous studies to compete favorably with tallow products, was used for comparison in these tests. Because suspensions of T-110 are viscous and tend to separate, T-110 was also tested in an aqueous-isopropyl alcohol formulation. Eighty grams of solid T-110 was melted at 70° C on a water bath and 20 grams of 80 percent isopropyl alcohol was stirred in. Upon cooling, this mixture was easily poured and dispersed in water. The final concentrations of isopropyl alcohol in the 0.25 and 0.5 percent formulations of T-110 were 0.05 and 0.1 percent, respectively.

Table 6 shows that at 1/2 pound 2,4-D per acre, the tallow products effectively enhanced the activity of the herbicide. Further, 0.5 percent T-110 (with isopropyl alcohol) and X-77 were significantly more effective than all other treatments except T-110 (without isopropyl alcohol). Table 7 shows similar results at 1.0 pound 2,4-D/acre, but not as many treatments were significantly different. This probably results from the large growth inhibition (64 percent after 14 days) caused by the high level of 2,4-D applied. Nevertheless, it is all the more remarkable that T-110 (with isopropyl alcohol) and X-77 at a concentration of 0.5 percent enhanced the effect of 2,4-D at highly significant levels of probability.

At the time the tests reported in Tables 6 and 7 were being made, a second test of phytotoxicity was made (Table 8). This was to recheck some previous results and to test for phytotoxicity of T-110 (with isopropyl alcohol) and X-77. As in the previous toxicity tests, no growth effects were significant and all surfactants used were non-phytotoxic as measured by growth of the cotton shoot.

# Field Trials in 1964

During 1964 a new tallow product became available, polyoxyethylenated sucroglyceride tallow (Ledoga Detergent Base Number One, LDB-1). This material is a liquid detergent and suspends T-110 in water. This suggested its use in formulations of sugar esters in herbicide sprays. To this end, the field trials of 1964 were to evaluate the use of LDB-1 in herbicide sprays, to verify certain of the results of 1963 tests, and to extend the work with T-110. The same methods and analyses used in 1963 were used (Tables 9, 10, and 11).

Sucroglyceride (T-110) again compared favorably with X-77 and Tween 20 at concentrations of 0.01 to 1.0 percent (Table 9). LDB-1 by itself effectively enhanced herbicide activity and the enhancement per unit of LDB-1 was about the same or slightly greater than that of T-110 (Tables 9 and 10). However, there appears to be an additive effect when T-110 and LDB-1 were applied together (Table 10). T-110 was most effective at 1.0 percent and increasing the concentration up to

## *Table 6. Effect of surfactants on the growth (dry weight in grams per 20 plants) and percent kill caused by 1/2 pound 2,4-D per acre*

There were nine replications of treatments 1 and 2 and five replications of all others.

Numbers following each mean indicate results of other treatments which are significantly different. Underlining of these numbers indicates differences which are highly significant.

| Treatment Number | Surfactant: Name | Surfactant: Concentration | Days after application: 7 | 14 | 21 |
|---|---|---|---|---|---|
| | | % | gram/20 plants | gram/20 plants | % kill |
| 1. | None and no 2,4-D | 0 | 36 | 47 | 0 |
| 2. | None | 0 | 22 1 | 20 1 | 1 |
| 3. | T-110 | 0.25 | 19 1 | 17 1 | 13 1,2 |
| 4. | T-110 | 0.50 | 16 1,2 | 14 1,2,9 | 37 1,2,7,8,9,10 |
| 5. | T-110 (with isopropyl alc.) | 0.25 | 17 1,2 | 15 1,2 | 20 1,2,7 |
| 6. | T-110 (with isopropyl alc.) | 0.50 | 17 1,2 | 13 1,2,9 | 41 1,2,3,5,7,8,9,10,11 |
| 7. | SET-1 | 0.25 | 19 1 | 16 1,2 | 5 1,2 |
| 8. | SET-1 | 0.50 | 18 1,2 | 15 1,2 | 8 1 |
| 9. | SETH-1 | 0.25 | 17 1,2 | 18 1 | 9 1,2 |
| 10. | SETH-1 | 0.50 | 18 1,2 | 14 1,2,9 | 15 1,2,7 |
| 11. | X-77 | 0.25 | 19 1,2 | 16 1 | 22 1,2,7,8,9,10 |
| 12. | X-77 | 0.50 | 18 1,2 | 12 1,2,7,9 | 57 1,2,3,5,7,8,9,10,11 |

## *Table 7. Effect of surfactants on the growth (dry weight in grams per 20 plants) and percent kill caused by 1.0 pound 2,4-D per acre.*

There were nine replications of treatments 1 and 2 and five replications of all others.

Numbers following each mean indicate results of other treatments which are significantly different. Underlining of these numbers indicates differences which are highly significant.

| Treatment Number | Surfactant Name | Surfactant Concentration % | Days after application 7 gram/20 plants | 14 gram/20 plants | 21 % kill |
|---|---|---|---|---|---|
| 1. | None and no 2,4-D | 0 | 53 | 58 | 0 |
| 2. | None | 0 | 29 1 | 22 1 | 2 |
| 3. | T-110 | 0.25 | 20 1,2 | 17 1,2 | 27 1,2 |
| 4. | T-110 | 0.50 | 21 1,2 | 17 1 | 31 1,2 |
| 5. | T-110 (with isopropyl alcohol) | 0.25 | 20 1,2 | 16 1,2 | 16 1,2 |
| 6. | T-110 (with isopropyl alcohol) | 0.50 | 17 1,2,7,8,10,11 | 16 1,2,7,10 | 41 1,2,5 |
| 7. | SET-1 | 0.25 | 24 1,2 | 20 1 | 21 1,2 |
| 8. | SET-1 | 0.50 | 23 1,2 | 16 1,2 | 34 1,2 |
| 9. | SETH-1 | 0.25 | 19 1,2 | 16 1,2 | 22 1,2 |
| 10. | SETH-1 | 0.50 | 23 1,2 | 19 1,2 | 32 1,2 |
| 11. | X-77 | 0.25 | 23 1 | 15 1,2,7,10 | 21 1,2 |
| 12. | X-77 | 0.50 | 19 1,2 | 14 1,2,7,10 | 39 1,2,5 |

## *Table 8. Effects of surfactants on the dry weight of cotton plants 14 days after application.*

All surfactants were at a concentration of 0.5 percent. No 2,4-D was in the sprays.

| Surfactant | Relative Difference of Dry Weight From the Control | p[1] |
|---|---|---|
| | % | |
| T-110 | −6 | 0.7 |
| T-110 (with 0.1 % isopropyl alc.) | −5 | 0.6 |
| SET-1 | +3 | 0.7 |
| SETH-1 | +1 | 0.9 |
| X-77 | −3 | 0.7 |

[1] Probability that the treatment is the same as the control (no surfactant).

## *Table 9. Effect of various surfactants on enhancing the growth effects of 2,4-D (1/16 pound per acre) on cotton plants. Field study in 1964.*

| Surfactant | Increase in dry weight after treatment | |
|---|---|---|
| Name, % conc. | 15 days | 31 days |
| | grams/20 plants | grams/20 plants |
| None and no 2,4-D | 79 a[1] | 398 a[1] |
| None | 67 ab | 249 b |
| T-110, 0.01 | 66 ab | 205 bc |
| 0.1 | 65 ab | 191 bc |
| 1.0 | 57 b | 165 c |
| T-110 and LDB-1, 0.01 and 0.01 | 63 ab | 223 bc |
| 0.1 and 0.1 | 58 b | 194 bc |
| 1.0 and 1.0 | 50 b | 161 c |
| LDB-1, 0.1 | 67 ab | 182 c |
| X-77, 0.01 | 66 ab | 207 bc |
| 0.1 | 65 ab | 212 bc |
| 1.0 | 57 b | 178 c |
| Tween 20, 0.01 | 68 ab | 201 bc |
| 0.1 | 55 b | 185 bc |
| 1.0 | 52 b | 158 c |

[1] Values followed by the same letter are not significantly different at the 1 percent level.

4.0 percent did not increase its effectiveness (Table 11). LDB-1 substitutes for isopropyl alcohol in the formulations containing T-110, maintains dispersion of T-110 and enhances the herbicide activity. But a mixture of T-110 and LDB-1 must be heated in order to disperse the T-110, even at 0.1 percent T-110.

## Table 10. Effect of a mixture of T-110 and LDB-1 on enhancing the growth effects of 2,4-D (1/16 pound per acre) on cotton plants. Field study in 1964.

| Surfactants | | Increase in Dry Weight After Treatment | |
|---|---|---|---|
| T-110 | LDB-1 | 21 days | 31 days |
| % | % | grams/20 plants | grams/20 plants |
| None and no 2,4-D | | 142 a[1] | 344 a[1] |
| 0.0 | 0.0 | 90 b | 164 b |
| 0.25 | 0.0 | 83 bc | 138 bcd |
| 0.00 | 0.25 | 76 bcd | 129 cde |
| 0.25 | 0.25 | 74 cd | 129 cde |
| 0.25 | 1.0 | 65 d | 118 cde |
| 0.25 | 4.0 | 62 d | 102 e |
| 1.0 | 0.0 | 91 b | 147 bc |
| 0.0 | 1.0 | 65 d | 112 de |
| 1.0 | 0.25 | 70 cd | 121 cde |
| 1.0 | 1.0 | 68 cd | 124 cde |
| 1.0 | 4.0 | 63 d | 104 de |
| 4.0 | 0.25 | 63 d | 108 de |
| 4.0 | 1.0 | 66 d | 95 e |
| 4.0 | 4.0 | 61 d | 100 e |

[1] Values in a column followed by the same letter are not significantly different at the 1 percent level.

# Conclusions

The results of field tests on cotton with sugar derivatives of tallow confirmed the prediction for their successful use which was based on greenhouse studies. Indeed, the tallow derivatives were as effective in herbicide sprays as the commercial surfactants used for comparison, and in several experiments the sugar-tallow derivatives were more effective than all but one of the commercial surfactants.

No phytotoxicity of the compounds was observed at any time in our studies, even when they were used at concentrations as high as 4 percent. Hence growth

## Table 11. Effect of a mixture of T-110 and LDB-1 on enhancing the growth effects of 2,4-D at various concentrations. Field study in 1964.

| Rate of 2,4-D | Surfactants | | Growth Effect After Treatment | |
|---|---|---|---|---|
| | T-110 | LDB-1 | Dry Wt. Increase After 12 Days | Kill After 21 Days |
| lb/acre | % | % | grams/20 plants | % |
| 0.0 | 0.0 | 0.00 | 142 a[1] | 0 a[1] |
| 0.25 | 0.0 | 0.00 | 65 c | 0 a |
| 0.25 | 0.1 | 0.10 | 64 cd | 1 a |
| 0.25 | 0.5 | 0.10 | 64 cd | 1 a |
| 0.25 | 1.0 | 0.10 | 58 cd | 1 a |
| 0.25 | 2.0 | 0.25 | 56 cd | 2 a |
| 0.25 | 2.0 | 0.50 | 58 cd | 1 a |
| 0.25 | 4.0 | 0.50 | 59 cd | 1 a |
| 0.50 | 0.0 | 0.00 | 75 b | 0 a |
| 0.50 | 0.1 | 0.10 | 59 cd | 12 b |
| 0.50 | 0.5 | 0.10 | 63 cd | 13 b |
| 0.50 | 1.0 | 0.10 | 52 d | 20 bc |
| 0.50 | 2.0 | 0.25 | 60 cd | 27 c |
| 0.50 | 2.0 | 0.50 | 61 cd | 24 c |
| 0.50 | 4.0 | 0.50 | 63 cd | 19 bc |

[1] Values in a column followed by the same letter are not significantly different at the 1 percent level.

responses of plants in the evaluation tests are attributable directly to the herbicide in the sprays.

T-110 (a sucroglyceride) was the most effective sugar-tallow surfactant used. However, it forms a viscous suspension when formulated at concentrations of 1 percent or more. A small percentage of isopropyl alcohol efficiently aids in decreasing the viscosity of the T-110 formulations and even increases the effectiveness of T-110 in enhancing the activity of 2,4-D. LDB-1 also maintains the dispersion of T-110 and is itself effective in enhancing the herbicide's activity. It is not known whether LDB-1 may be safely consumed by animals; its safe use in agricultural sprays remains to be evaluated. Therefore, it is our recommendation at this time that T-110 be dispersed by isopropyl alcohol or similar solvent which will volatilize shortly after spray application.

All studies by others to date have shown that sugar esters of fatty acids normally found in tallow are harmless to animals, even when eaten. In fact, sugar esters may be used as a major dietary constituent of those animal species tested so far. In view of these findings, sugar esters as surfactants in pesticide sprays are competitive with most agricultural surfactants now being used, and offer the great advantage of being edible.

Although all sugar esters tested were effective adjuvants in herbicide sprays, complex sucroglycerides such as T-110 show greatest potential for use in herbicide and pesticide sprays. Many more trials under a broad range of conditions are needed, of course, before general applicability of sugar esters in agricultural sprays may be properly assessed. But along with their edibility, low cost, and commercial availability, our results suggest great promise for them.

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*Part II*

# THIN-LAYER CHROMATOGRAPHY OF SUGAR ESTERS AND SUCROGLYCERIDE[A, B]

Stephen B. Bingham and Edwin B. Kurtz, Jr. [C]

In preliminary studies with sugar esters as surfactants, mono-esters were prepared in the laboratory and they were assayed by hydrolysis and determination of molar proportions of sucrose and fatty acid moieties (6). However, this method of analysis did not provide information about the presence of free sucrose and di-esters of sucrose and no information was obtained about the 1 to 3 percent of impurities. Also, as the field studies became involved with the use of commercial sucrose tallowates and a sucroglyceride, the need for a qualitative and semi-quantitative analysis of these complex mixtures became apparent (3): At about that time Gee published a paper (2) on thin-layer chromatography of sucrose esters. This method was tested and subsequently modified to permit analysis of sucrose tallowates and sucroglyceride. The latter requires two-dimensional thin-layer chromatography and the methods are described in this paper. The general technique of thin-layer chromatography is described elsewhere, for example (1). It is hoped that the procedures described in this paper will provide those who undertake research with sugar esters and sucroglyceride with a simple, rapid, and adequate analysis of these complex mixtures.

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[B] The mention of commercial products and companies anywhere in this paper does not imply that they are endorsed or recommended by the Arizona Agricultural Experiment Station over others of a similar nature not mentioned.

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# Experimental and Results

**One-dimensional chromatography of sucrose esters.** Glass plates 200 mm long were coated with a layer of silica gel 0.3 mm thick. Pure silica gel was used rather than silica gel containing a $CaSO_4$ binder as was used by Gee (2). The plates were spotted with 10 to 500 µg of tallow product and were developed for approximately one hour in 200 ml of solvent in a one gallon jar covered with a glass plate. The developing solvent was toluene: ethyl acetate: 95% ethyl alcohol (2:1:1 by volume) as used by Gee (2). Another solvent may be used (5% methanol in benzene: 10% isopropyl alcohol in benzene, 1:1 by volume), but the former solvent gives better resolution of spots.

After developing the plates, they were air-dried and the spots detected by spraying the plates with a 50:50 aqueous solution of $H_2SO_4$ and charring in an oven at 110° C. The relative density and the size of the charred spots were used as a semi-quantitative estimate of the components in the tallow product (Figure 1). Spots may be detected also by spraying the plates with a 0.5% solution of 2′, 7′-dichlorofluorescein in methanol as described by Gee (2), but we found that smaller quantities of components may be detected by the use of $H_2SO_4$ and charring.

Identification of components on the chromatograms was done as follows with SEO-1 (sucrose mono-oleate, purified, Colonial Sugars, Inc.) as the test mixture. A total of 30 mg of SEO-1 was spotted onto six plates and developed as described. One edge of each plate was sprayed with 2′, 7′-dichlorofluorescein to locate the spots. Using these spots as references, the silica gel from each of five regions on the plate (Figure 1) was scraped off. The components in each region were eluted from the silica gel with 4 to 10 portions of redistilled dimethylformamide and filtered under vacuum through cintered glass. Fractions were evaporated to dryness in tared flasks under vacuum at 75° C. The residue from each fraction was dissolved in chloroform. A small portion was rechromatographed and another small portion was tested with hydroxylamine hydrochloride for the presence of ester bonds (5). The remaining known quantity of each fraction was hydrolyzed with 4.0 ml 10% HC1 on a hot water bath for 2 hours, diluted with water and extracted with petroleum ether (density 0.69 - 0.70). The petroleum ether and water phases were separated and evaporated to dryness in tared beakers and weighed. The residue from the water-soluble fraction was chromatographed on silica gel by thin-layer chromatography using a solvent of toluene: ethyl acetate: 95% ethyl alcohol (1:1:2 by volume). Sucrose, glucose, and fructose were also chromatographed as reference compounds. The residue from the fraction which was soluble in petroleum ether was chromatographed on silica gel plates which had been saturated with 5% silicone in petroleum ether and dried. The plates were developed in 75% aqueous acetic acid for 3 to 4 hours (4). Known fatty acids also were chromatographed as references. Spots on both kinds of plates (sugars and fatty acids) were located by spraying with 50:50 aqueous $H_2SO_4$ and charring at 110° C.

Region 1 from the SEO-1 plates contained sucrose. Region 2 contained largely the mono-ester of sucrose, although traces of glucose and fructose were also present. Region 3 contained the di-ester of sucrose; the molar ratio of fatty acid to sucrose was 2:1. Region 4 contained sucrose combined with the fatty acid, but there was insufficient material to determine the molar ratio of fatty acid to sucrose. This region is believed to contain the tri-ester of sucrose. Region 5 contained the methyl

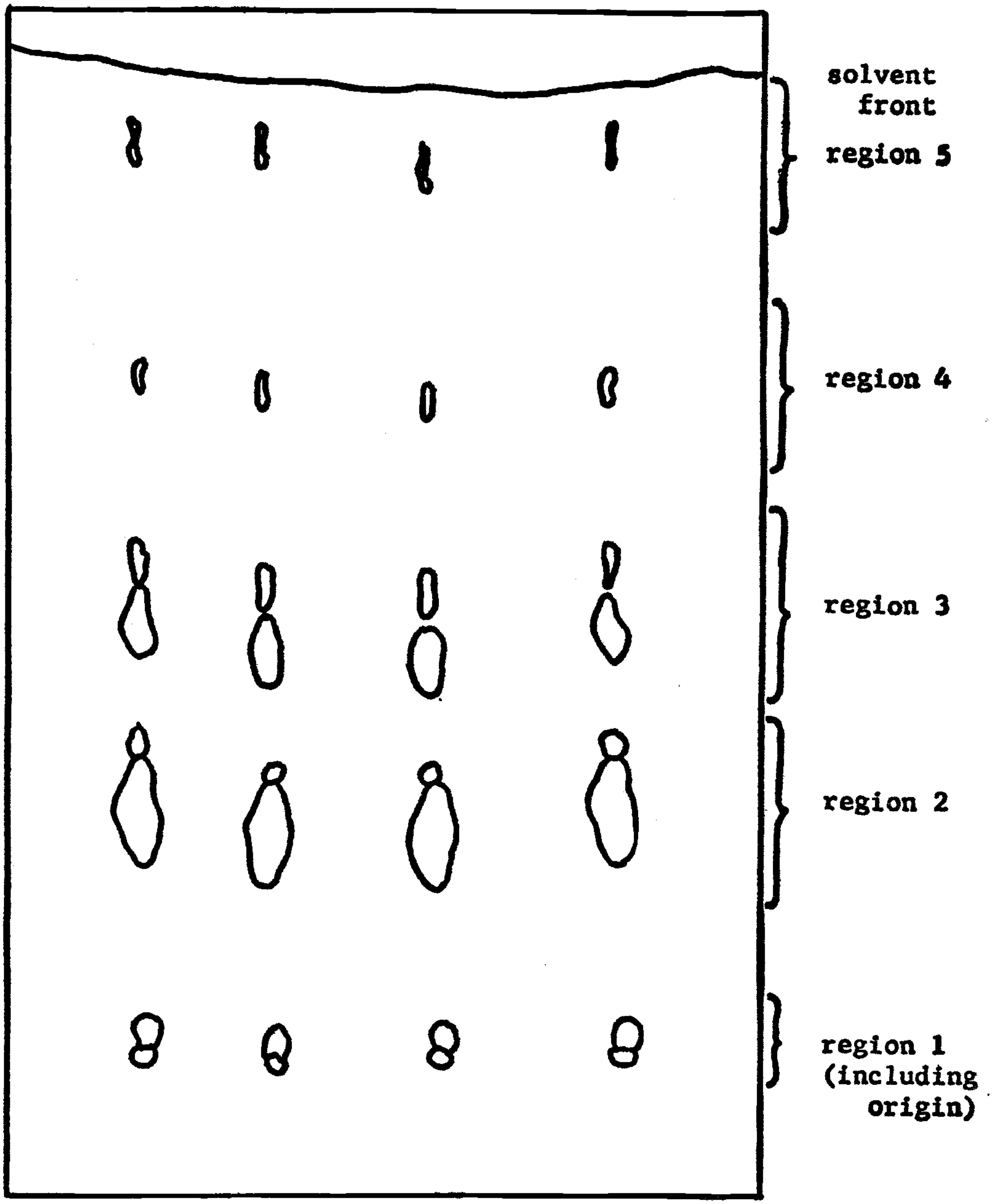


Figure 1. Separation of SEO-1 (sucrose mono-oleate) by one-dimensional thin-layer chromatography. This figure is a tracing of the plate after detection of the spots by spraying with $H_2SO_4$ and charring. Numbers denote regions removed for analysis (see text). Main constituents in each region are: 1, sucrose; 2, sucrose mono-ester of oleic acid; 3, sucrose di-ester of oleic acid; 4, sucrose tri-ester of oleic acid; 5, methyl ester of oleic acid.

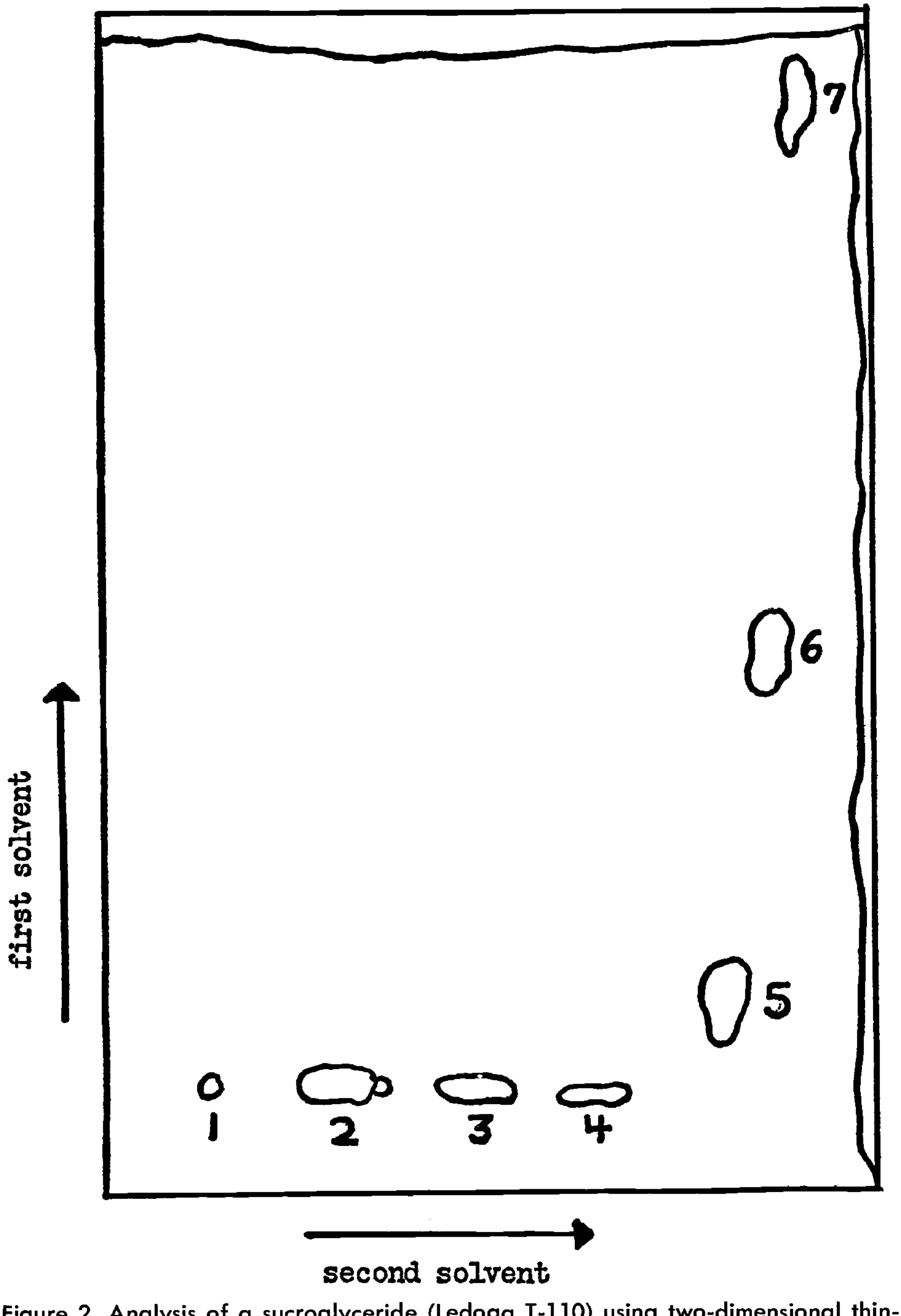


Figure 2. Analysis of a sucroglyceride (Ledoga T-110) using two-dimensional thin-layer chromatography. This figure is an actual tracing of the plate after spraying with $H_2SO_4$ and charring. The spots were identified as follows: 1, origin and sucrose; 2, mono-esters, 3, di-esters, and 4, tri-esters of sucrose and fatty acids from tallow; 5, mono-glycerides; 6, di-glycerides; and 7, tri-glycerides of tallow.

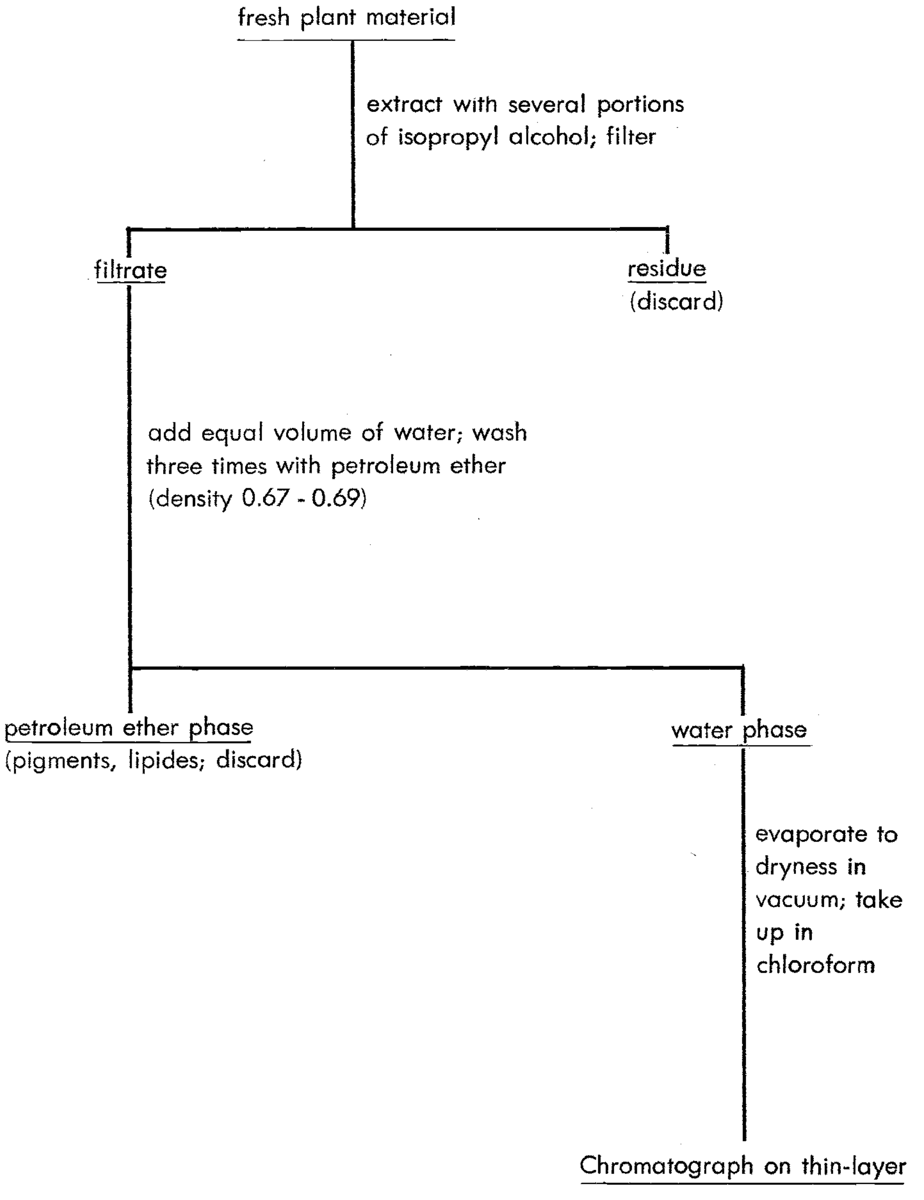


**Figure 3. Procedure for the extraction and identification of sucrose esters from plant material to which the esters have been applied.**

ester of the fatty acid, presumably the unreacted ester remaining from the commercial preparation of the sugar ester.

**Two-dimensional chromatography of sucroglyceride.** The sucroglyceride T-110 (Ledoga, S.p.A., Milano, Italy) was analyzed by spotting 100 µg, T-110 dissolved in chloroform onto plates covered with a 0.3 mm layer of silica gel. No binder was used. The plates were developed for one hour with a solvent (5) of petroleum ether (density 0.67 - 0.69):diethyl ether (70:30 by volume). The plates were dried and developed in the second dimension with a solvent (2) of toluene:ethyl acetate:95% ethyl alcohol (2:1:1 by volume). The plates were dried, sprayed with 50:50 aqueous $H_2SO_4$, and charred at 110° C (Figure 2). Spots were identified as described for one-dimensional chromatography.

Using density and size of the charred spots to semi-quantitatively estimate the components, the analysis of sucroglyceride T-110 is:

25% mono-esters of sucrose
12% di-esters of sucrose
12% tri-esters of sucrose
30% mono-glycerides
12% di-glycerides
8% tri-glycerides
trace sucrose

**Recovery of sucrose esters from plant material.** In connection with the chromatographic analyses of sucrose esters, a procedure was developed for the quantitative recovery of sucrose esters from plants which had been sprayed with these materials (Figure 3). Eighty to 100 percent of the sucrose esters sprayed on plants were recovered by this procedure. No sucrose esters were detected in untreated bean plants.

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