12.1 μg/kg for soybean oil in the samples they analyzed. The source of PAH in the oils is presumably from initial environmental contamination of the vegetables with the derived oils essentially retaining the PAH.

CONCLUSION

From a survey of selected food items from plant or dairy origin, it appears that Canadians are subjected to similar levels of PAH as reported in other countries. Wheat bran products seem to be significantly higher in PAH content than other types of cereal grain foods. Some vegetable oils may also contain low levels. Processing of certain food items by direct heating methods can lead to a significant increase in PAH content of the finished product, depending upon the type of fuel used, the combustion conditions, and the type and degree of exposure of the food to the flue gases. Some correlation between PAH and nitrosamine levels in direct dried milk products and malt was found.

Registry No. NDMA, 62-75-9; FL, 206-44-0; PY, 129-00-0; BbFL, 30777-19-6; DMP, 1576-67-6; BaA, 56-55-3; Per, 198-55-0; BaP, 50-32-8; DacA, 215-58-7; DahA, 53-70-3; Pi, 213-46-7; IP, 193-39-5; DaeP, 192-65-4; An, 191-26-4; DPA, 1499-10-1.

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Rapid Isolation and Thin-Layer Chromatographic Screening of Extracts from Boll Weevil (*Anthonomus grandis* Boheman)

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A rapid isolation procedure is described for producing separate lipoidal and polar component extracts from small samples of boll weevils. Tissue grinding and extraction were done simultaneously in a capsular mortar in a high-speed triturating device. The extracting solvent of 2:1 chloroform–methanol and pulverized tissue were transferred from the capsule to a microcentrifuge tube, and water was added to induce phase separation, which was aided by centrifugation. Both extracts were analyzed by thin-layer chromatography on silica gel; the developing solvent was hexane–ethyl ether–acetic acid (90:10:1 v/v) for the chloroform extract and benzene–acetic acid–water–nitromethane (60:40:10:30 v/v) for the aqueous methanol extract. The utility of the method was illustrated by the ability to differentiate individual samples of young and old boll weevils of both sexes taken at different times during the year. Samples differed in the amount of lipoidal and polar components they contained.

Since the pioneering work of Reiser et al. (1953) on the lipid composition of the boll weevil (*Anthonomus grandis* Boheman), increasingly more sophisticated and detailed studies have been made in an effort to gain understanding of the relationship of the insect's lipid content and its physiological state (Brazzel and Newsome, 1959; Guerra et al., 1982). In spite of these efforts, the biochemical regulation of these physiological states, especially diapause, remains, for the most part, unknown.

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In more recent chemical studies (Lambremont et al., 1964; Joiner and Lambremont, 1969; Keeley et al., 1977) workers have utilized modifications of the lipid isolation method of Folch et al. (1957) and conducted analyses of the isolates by either column, gas, or thin-layer chromatography. The essential features of their modified methods include repetitive homogenization and solvent extraction, followed by phase separation over an extended time at low temperature. Although the original and modified isolation methods meet the needs of the analytical scheme for which they were developed, they are too tedious and time consuming for the frequent processing of a large number of small samples (one to five boll weevils) where only semiquantitative results are sought.

The regulatory roles that lipids and other components play in weevil metabolism and physiology are not known

in detail. Because this information is needed to determine the existence of diapause in feral weevils from subtropical and tropical areas, there is a need to develop more practical methods to isolate and assay these components in weevil tissue. Therefore, our purpose was to develop and report here a practical and rapid method of isolating separate lipid and aqueous fractions from small samples of boll weevils and a scheme of thin-layer chromatographic analysis of these extracts for assaying lipodal and water-soluble components of possible physiological importance. Use of the method is illustrated by application to various samples of boll weevils.

MATERIALS AND METHODS

A sample consisting of five boll weevils (ca. 75 mg total), either frozen or alive, was placed in a 2-mL Teflon capsule (E-Z Kleen model, The Toothmaster Co., Racine, WI). This capsule serves as a mortar in a high-speed triturating device such as those used by dentists for preparing amalgams. A plastic cylindrical pestle is placed in the capsule on top of the insects and 0.5 mL of a 2:1 chloroform–methanol extracting solvent (Folch et al., 1957) was added (solvents were "Baker Resi-Analyzed" reagent grade). The capsule was capped and then shaken for 15 s on the high-speed triturating device (Wig-L-Bug, Model DS-80, Crescent Dental Manufacturing Co., Lyons, IL). After trituration, the extracting solvent was decanted from the capsule into a 1.5 mL capacity polypropylene microcentrifuge tube. A second 0.5-mL volume of extracting solvent was added to the capsule and trituration was repeated. After the second trituration, the extract and pulverized tissue were transferred to the microcentrifuge tube with the aid of a microspatula. Further rinsing of the emptied capsule and pestle with additional extracting solvent during a third shaking on the triturating device was found to be unnecessary since analysis of that rinse showed it to contain only negligible amounts of extractable components. The capsule and pestle were cleaned between runs by rinsing 3 times with the solvent mixture and then wiping dry with laboratory tissue. No detectable contaminating components were found in an extracting solvent blank that had been run with the cleaned capsule and pestle and carried through the entire procedure.

Distilled water (0.2 mL) was added to the microcentrifuge tube containing the sample and the tube capped and then briefly shaken prior to centrifuging in an Eppendorf Micro Centrifuge (Model 5412, 15600 *g*'s) for 1 min. After centrifugation, the pulverized boll weevil tissue was mainly located as a thin compacted layer between the lower chloroform phase and the upper aqueous methanol phase. This compacted layer has sufficient structural integrity so as to be easily removed as a unit with a microspatula. Additional pulverized tissue was present as a small pellet at the tip of the tube below the chloroform layer. Layer partition was well defined with no evidence of emulsification. Tubes were placed in −20 °C storage until needed for thin layer chromatographic analysis.

The chloroform was analyzed for lipoidal components by thin-layer chromatography on silica gel (silica gel 60 HP-TLC, EM Laboratories, Elmsford, NY) with hexane–ethyl ether–acetic acid (90:10:1 v/v) as a developing solvent (Mangold, 1969) (solvents were "Baker Analyzed" HPLC reagent grade). The chloroform phase of each sample (50 μL) was spotted on the plate along with a mixture of lipid standards (Non-polar Lipid Mix B, Supelco, Bellefonte, PA). Eighteen samples could be chromatographed simultaneously on a 20 × 20 cm plate. Components of the individual lipid classes separated on the plate were visualized by viewing on a 365-nm ultraviolet transilluminator (Ultra-Violet Products, Inc., San Gabriel, CA) and also by exposing the plate to iodine vapor in a closed chamber and viewing in visible light. No detectable lipid components were observed in the aqueous methanol extract when chromatographed with the same system (Figure 1).

In a similar manner a second silica gel TLC plate was prepared with separate 50-μL volumes of the aqueous methanol and chloroform extracts. This plate was developed in a solvent mixture of benzene–acetic acid–water–nitromethane, 60:40:10:30 v/v (nitromethane was "Fisher Certified" reagent and the other solvents were "Baker Analyzed" HPLC reagent grade). We formulated this solvent system to give the optimum separation of components found in the aqueous methanol extracts. Visualization of the separated components on the developed plates was accomplished by the following sequence: (1) exposed to ammonia vapors and then viewed and photographed in 365-nm ultraviolet light; (2) exposed to iodine vapors and then viewed and photographed in visible light; (3) sprayed with 2′,7′-dichlorofluoroscein (0.2% in 2-propanol), then heated to 125 °C for 5 min, and then viewed and photographed in both 365-nm ultraviolet and visible light.

Rough estimates of the relative amount of each lipid class, within and between samples, could be made by visually comparing spot size and density with those of the component spots of the standard lipid mixture of known concentration. When 15 μL of this standard mixture was spotted, 357 μg of each of its five components was present. Since this amount was intermediate in the range of some of the corresponding spots in the extracts, those standard spots were assigned an arbitrary value of 3, and multiples and submultiples of 15 μL of the standard could be used for approximating other values. Similar relative values for the unknown fluorescent components in the aqueous methanol extract could be assigned relative to some suitable standard. These estimates could be made either with the original TLC plates or with the photographs.

To test the validity of the method, individual boll weevils were collected from traps in the vicinity of Brownsville, TX (~26° north latitude) during the early spring of 1980 (April 18) and 1981 (March 6, 9, and 15; April 20). The weevils were divided into four groups: (1) young male, (2) young female, (3) old male, and (4) old female. Here young are defined as being less than 3 days old, as evidenced by the reddish coloration and softness of the body. The sexes were differentiated by examining the posteroventral portion of the abdomen so that the last tergum and sternum can be observed. The male has a characteristic notch in the last tergum (Agee, 1964). These four groups of weevils collected on the above dates were subjected to component profile comparisons by the rapid screening procedure.

Weevils of known age that were reared exclusively on cotton squares (a specific anatomical term for cotton flower buds) were also analyzed. Squares obtained from a weevil-infested field during early june 1981 were brought to the laboratory, and young weevils were collected as they emerged from the squares. These weevils were supplied daily with fresh squares to feed on during their development. Analyses were made on separate samples of the male and female weevils (five individuals per sample) taken at 1, 5, and 20 days after they emerged from the original squares.

RESULTS AND DISCUSSION

Among weevils of both sexes collected in early April 1980, it was observed that more of an unidentified yellow fluorescent component was present in the aqueous methanolic extracts from the old weevils than in those from the

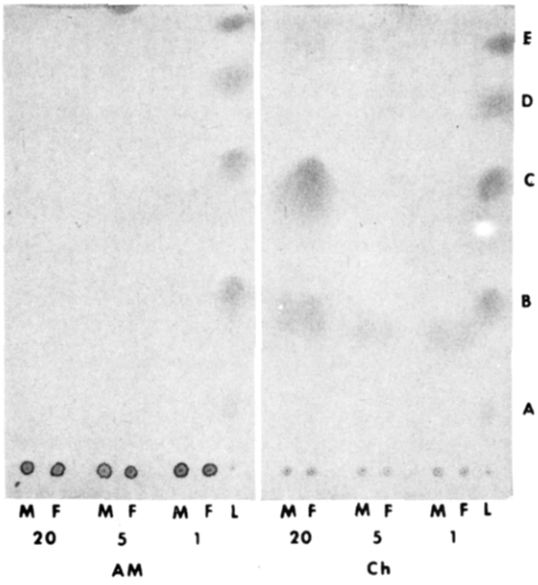

Figure 1. Thin-layer chromatograms of aqueous methanol (AM) and chloroform (Ch) extracts of male (M) and female (F) boll weevils at 1, 5, and 20 days after they emerged from cotton flower buds (squares). Lipid standard (L) contains (A) cholesterol, (B) oleic acid, (C) triolein, (D) methyl oleate, and (E) cholesterol oleate. The developing solvent was hexane–diethyl ether–acetic acid, 90:10:1 v/v. The chromatograms were photographed in visible light after exposure to iodine vapor.

young weevils. Two other fluorescent components were found in the extracts from old weevils that were not detected in those from young weevils.

In the chloroform extracts of these same weevils, a blue fluorescing component was more prominent in the extract of old females than in that of young females. The same component was also found to be present in greater amounts among old males than in young males but in amounts less than that of the females of comparable age. Both the male and female old weevils contain a fluorescent component that is lacking in the young weevils.

The aqueous methanol extracts of the weevils collected in mid-March 1981 contained considerably more blue fluorescing components than were observed in those extracts collected at the other times during the year. This same extract from the female weevils collected in early March contained a component in considerable excess of that present in all the other extracts.

Four of the five major components found in the chloroform extracts of these same weevils cochromatographed with the triglyceride triolein, the free fatty acid oleic acid, the steriod cholesterol, and the sterol ester cholesterol oleate. Weevils collected in late April had more of the component at the triolein position than did those weevils collected in mid-March. Males collected in early March had considerably more of that same component than females collected at the same time.

The right half of Figure 1 is the TLC plate of the chloroform extracts of the weevils of known age that were reared exclusively on cotton flower buds (squares). The triglyceride component increased between the 5th and 20th day of development. This increase was considerably greater in the female than in the male. The free fatty acid component appears to about double between the 5th and 20th day for both the male and female weevils. The relative amounts of the lipids found in these weevils are summarized in Table I.

In Figure 2 can be seen the large number of fluorescent components which are present in both the chloroform and aqueous methanol extracts of the weevils reared on cotton

Table I. Relative Amounts[a] of Lipids in Square-Fed Boll Weevils

lipid class	sex	days after emerging from squares: 1	5	20
fatty acids	M	1	1	2
	F	1	1	2
triglycerides	M	0	0	2
	F	0	0	5
sterol esters	M	T	0.3	0.6
	F	T	0.3	0.6

[a] T = trace. A value of 3 is approximately equal to 357 μg of the lipid component.

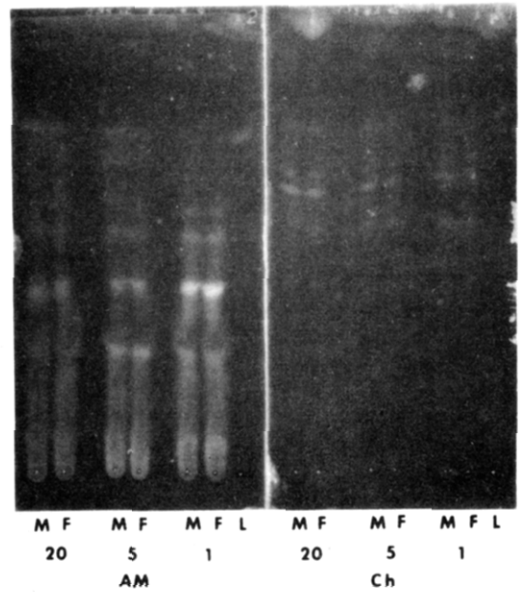

Figure 2. Thin-layer chromatograms of aqueous methanol (AM) and chloroform (Ch) extracts of male (M) and female (F) boll weevils at 1, 5, and 20 days after they emerged from cotton flower buds (squares). The developing solvent was benzene–acetic acid–water–nitromethane, 60:40:10:30 v/v. The chromatograms were treated with ammonia vapor and photographed while exposed to long-wave ultraviolet light.

squares. Whereas the amounts of some of these components increase with advancing physiological development, other components decrease. Sex differences are less apparent.

Keeley et al. (1977) makes the point that although lipid content is the most widely used criterion for determining boll weevil diapause, it has proven to be inconclusive with insects collected from a tropical environment. He concludes that only when the lipid content of diapause and nondiapause individuals collected at the same tropical location were compared directly were the two physiological states distinguishable. In subtropical regions, boll weevils may live for as long as a year and during any time of the year there are always some that remain active.

The use of the rapid screening technique described here may provide a tool to allow such comparisons to be easily made on a semiquantitative basis without the use of sophisticated and expensive equipment. The added advantage of utilizing the aqueous methanol extract in the screening is that it may provide additional markers of the insect's physiological state or provide indirect evidence for the nature of the insect's diet. The solvent system utilized for the separation of components in the aqueous methanol extract has been optimized to provide a capability of separating components not only of the boll weevil but also of food components in its digestive tract. This capability may provide a useful tool for determining possible alter-

nate food sources of the boll weevil in a tropical environment. When components are found that may correlate with either the physiological state of the insect or its food source, efforts would be needed to make chemical identification of such markers.

Registry No. Triolein, 122-32-7; oleic acid, 112-80-1; cholesterol, 57-88-5; cholesterol oleate, 303-43-5.

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Behavior of Pyrethroid Insecticides under Liquid–Solid Chromatographic Conditions

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A series of pyrethroid insecticides and associated carbonyl compounds was chromatographed. A high-performance liquid chromatographic system equipped with an infrared detector was used with a 5-μm silica gel column that was eluted with either a ternary solvent mixture composed of acetonitrile, dichloromethane, and heptane or a binary mobile-phase system composed of 1-tetradecene in dichloromethane. The logarithm of the capacity factor (k') was found to be linearly correlated with the logarithm of the mole fraction (N_b) of acetonitrile or 1-tetradecene in the respective mobile phase. The slope and y-intercept values of the graphs obtained from log k' vs. log N_b were discussed in terms of the molecular structure and the biological activity of the investigated compounds.

Basically, two physical models have been proposed for the mechanism of retention in liquid–solid chromatography (LSC). They are the competitive displacement model (model I) formulated by Snyder (1968, 1974), Soczewinski (1969, 1977), and Soczewinski and Golkiewicz (1973) and the solvent interaction or sorption model (model II) proposed by Scott (1976) and Scott and Kucera (1975, 1978). Snyder (1974) and Snyder and Poppe (1980) compared the two models and provided a critical and comprehensive evaluation of their respective advantages and limitations.

Practical applications of these models in LSC are the planning of adequate separation procedures for the analysis of certain component mixtures, the prediction of retention data, and the formulation of quantitative structure–retention relationships that can be used in identifying the different components of the chromatographed samples. An attempt will be made in the present investigation to use the LSC structure–retention relationship data in structure–bioactivity relationship studies of a major class of synthetic insecticides, the pyrethroids. The pyrethroid insecticides are generally divided into two major categories, the "knockdown" and the "killing" agents. It has been reported (Briggs et al., 1974) that the important property that determines whether a particular pyrethroid will have good knockdown or killing activity is the overall polarity of the pyrethroid molecule and that the optimum polarity for knockdown effect is greater than the polarity required for killing action. The explanation for the difference in polarity requirements between knockdown and killing activities was based on the assumption that the knockdown effect depends on relatively rapid penetration of the pyrethroid through the insect cuticle and into the hemolymph and hence faster arrival at the site of action, the central nervous system. It has also been suggested (Briggs et al., 1976) that both rate of penetration and susceptibility of the compound to detoxification depend upon the polarity of the pyrethroid molecule. However, no evidence was reported to show that knockdown agents penetrate faster or that they are detoxified faster. Partition coefficient values determined between nitromethane and petroleum ether for a series of pyrethroid compounds gave essentially no correlation to the knockdown index obtained with *Musca domestica* (Lee, 1976). Also, partition coefficient values of different pyrethroids estimated by using reversed-phase thin-layer chromatography showed no correlation with bioactivities, whereas the size, shape, and electronic effects of the pyrethroid molecules gave good correlations with bioactivities. From structure–activity relationship studies of two congeneric series of pyrethroids by using multiple regression analysis of bioactivity vs. substituent constants (Hansch's π values and Hammett's σ constants), different optimum π values were indicated for knockdown and killing toxicity (Ford, 1979). However, it was suggested that the difference in polarity require-

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