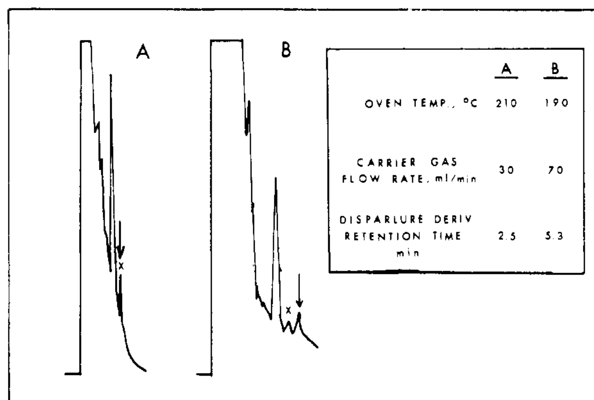

Figure 3. Separation of a background peak from the disparlure derivative peak in chromatograms of an air sample taken from a disparlure-treated Maryland field. The arrows indicate the derivative peak and the crosses indicate the background peak.

of concentrations in air are shown in Table I. Air flow through the system in individual tests ranged from 3.6 to 6.5 m^3, corresponding to disparlure concentrations in the fortified air ranging from 2.7 to 506 ng/m^3. Recoveries among replicates varied considerably, but part of the variability was undoubtedly caused by the experimental technique, which involved a material balance for the entire system. Results for field samples would therefore be expected to be less variable, with slightly higher recoveries than those for laboratory samples.

With both field air and prescrubbed laboratory air, as much as 30 m^3 of air were drawn through the system without appreciable effect on the cleanliness of the chromatograms or on disparlure recovery. This shows not only that the molecular sieves strongly sorb the chemical, but also that field air does not contain components that in themselves, or possibly in reaction with triphenylphosphine dibromide, are sensitive to electron-capture detection. In one set of analyses of forest air that contained very low levels of disparlure, we were able to quantitate only 6 ng of the chemical in 30 m^3 of air, so that the limit of quantitation of the method is about 0.2 ng of disparlure/m^3 of air.

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Determination of Antibiotic Residues of Plauracin in Swine Tissues

Hassan G. Fouda* and Martin J. Lynch

A bioautographic method is described for the quantitative determination of the antibiotic complex plauracin at levels down to 20 ppb in edible tissues of swine. The method involves extraction, partial purification, concentration, and application to silica gel plates followed by incubation with *Sarcina lutea* seeded agar. Measuring areas of inhibition zones by the method of Xerox copying provided a basis for quantitation. A group of 16 pigs were maintained on plauracin in feed at 110 ppm for 29 days. Following withdrawal of the medication, groups of three pigs were slaughtered at timed intervals, and edible tissues of each were analyzed for plauracin residues by the method described. With no withdrawal of the antibiotic, fat contained 25 ppb plauracin, and was the only tissue showing measurable (>20 ppb) activity. One day after withdrawal of the medication, measurable residues were not present in fat or any of the major edible tissues.

Plauracin is a novel antibiotic complex produced by a new strain of *Actinoplanes* (*Amorphosphorangium*) *auranticolor* (Celmer et al., 1975). It belongs to the same family as PA-114, virginiamycin, mikamycin, and others. Each of these is a combination of several macrocyclic lactone and depsipeptide components. Separately, each component exhibits a bacteriostatic effect, but in combination they are synergistic and bactericidal. Preliminary indications are that plauracin possesses good growth promoting and antidysentery activities combined with a low toxicity profile. It is presently under evaluation as a feed additive for growth promotion of swine and for the prophylaxis and treatment of swine dysentery.

A tissue residue method for the analysis of the antibiotic virginiamycin in swine tissue has previously been described (DiCuollo et al., 1973). In our hand, efforts to extend the limit of detection of this method to levels below 0.1 ppm were not successful. We report here the development of a sensitive microbiological assay for plauracin antibiotic residues in swine tissues. Application of the method to

Central Research, Pfizer Inc., Groton, Connecticut 06340.

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investigate the potential of plauracin to produce antibiotic residues in tissues of swine fed the drug at the maximum projected use level is also described.

MATERIALS AND METHODS

Chemicals and Supplies. Plauracin, Lot No. 8457-32-2F with an assigned potency of 100%, was obtained from Pfizer, Inc., Groton, Conn. Nunc bioassay dishes 23 × 23 cm were obtained from vangard International Inc., Red Bank, N.J. Willems-Polytron homogenizer, Model PT-20 equipped with sawtooth generator PT-20ST, was obtained from Brinkman Instruments, Westbury, N.Y. Silica gel TLC plates 20 × 20 cm, 250 μm were also obtained from Brinkman Instruments and were acidified by dipping in 2% methanolic acetic acid solution, followed by reactivation at 100 °C for 30 min. Klett-Summerson photoelectric colorimeter was from Klett Manufacturing Co., New York, N.Y. All solvents were glass distilled, Burdick & Jackson or equivalent, and all reagents were ACS grade.

Animal Studies. Sixteen swine, eight females and eight castrated males weighing between 70 and 90 kg, were maintained for 29 days on plauracin-medicated feed. The medicated feed was prepared by the homogenous mixing of 100 g of the fermentation product with each short ton of the swine basal ration. The basal ration was composed of ground corn (85.95%), soybean meal (10.9%), dicalcium phosphate (1.65%), ground limestone (0.4%), iodized salt (0.5%), vitamin premix (0.5%), and trace mineral premix (0.1%). The final medicated feed was assayed at 108 ± 5.3 ppm. This level of medication is equal to or greater than the projected use level. The animals were group fed. Their average feed consumption and weight gain were noted. At the end of the 29-day feeding period, zero withdrawal, three pigs were slaughtered. The remaining animals were withdrawn from the medicated feed and placed on the nonmedicated basal ration.

Groups of three pigs each were slaughtered at 1, 2, and 3 days following drug withdrawal. Liver, muscle, kidney, and fat of the slaughtered animals were excised and frozen immediately. Samples of the frozen tissues were used for the assay of plauracin content.

Extraction of Plauracin from Tissues. Tissues were cut into small pieces and 20-g samples were weighed into 250-mL centrifuge bottles. To liver, muscle, and kidney was added 20 mL of 0.1 M phosphate buffer at pH 6, and the tissues were homogenized for 1 min with 60 mL of ethyl acetate using a Willems-Polytron homogenizer. The homogenate was centrifuged and the supernatant ethyl acetate extract was decanted. The remaining material was reextracted with 30 mL of ethyl acetate two successive times. The combined extract was filtered through anhydrous sodium sulfate into a 250-mL round-bottom flask and was evaporated to dryness by rotary vacuum evaporator. The residues were transferred quantitatively with 5 mL of acetonitrile to a 50-mL centrifuge tube. The acetonitrile extract was partitioned three times with 10 mL of hexane previously saturated with acetonitrile. The hexane layers were discarded and the purified acetonitrile layer was then diluted with 35 mL of distilled water. The aqueous solution was extracted three times with 10 mL of ethyl acetate. The combined ethyl acetate extract was filtered through anhydrous sodium sulfate into 50-mL centrifuge tubes and was evaporated to dryness by a nitrogen stream. The residues were dissolved in 0.4 mL of acetone and 10-μL aliquots of this acetone extract were used for the assay of plauracin.

Fat was homogenized three times with 30 mL of acetonitrile. The combined extract was transferred to a separatory funnel and was partitioned three times with 90 mL of hexane previously saturated with acetonitrile. The hexane layers were discarded but the bottom acetonitrile layer was transferred to a 250-mL round-bottom flask and concentrated to about 2 mL using a rotary evaporator. The remaining acetonitrile was quantitatively transferred with 20 mL of ethyl acetate to a 50-mL centrifuge tube and 20 mL of 10% sodium chloride was added. The tube contents were shaken vigorously, and after the separation of phases, the ethyl acetate layer was saved and the aqueous layer was extracted two additional times with 5 mL of ethyl acetate. The three ethyl acetate layers were combined, filtered through anhydrous sodium sulfate, and evaporated to dryness under a gentle nitrogen stream. The residues were dissolved in 0.4 mL of acetone, and 10-μL aliquots of this acetone extract were used for assay.

Preparation of Assay Inoculum and Medium. The assay organism *Sarcina lutea* ATCC 9341 was maintained on full-strength Brain Heart Infusion. The inoculum was prepared by washing a 24-h slant with 10 mL of sterilized saline solution. The suspension was centrifuged for 15 min and the supernatant was decanted. The cells were suspended in saline solution and then diluted until a density of 300 Klett units was obtained on a Klett-Summerson photoelectric colorimeter employing a red filter (660 nm). The inoculum, thus prepared, was found to be viable for at least 1 week when stored at 4 °C. The inoculated medium was prepared by melting 170 mL of agar on a steam bath, cooling it to 48 °C in a water bath, and then adding 1 mL of the assay inoculum with gentle swirling.

Preparation of Standard Curves. Separate standard curves were prepared for each tissue to eliminate the effects of tissue background on the diffusion of the drug. For studying the recovery of plauracin from tissues the method of external standardization was used. In this case, extracts of control tissues were prepared according to the previously described method and a standard solution of plauracin (1 mg/100 mL) in acetone was diluted with the control tissue extracts for construction of each standard curve. Since preliminary investigation showed the assay to be linear only in the range of 10–40 ng/10 μL, the standard curve concentrations were constructed over this range and were equivalent to 20 to 80 ppb of plauracin in tissue. Extracts of tissues fortified with 100 ppb were diluted in half to assay approximately at the midpoint of the standard curve. For the analysis of samples of medicated tissues, the method of internal standardization was followed. In this case 20-g portions of control tissues were fortified with plauracin at levels equivalent to 0, 20, 40, 60, and 80 ppb. The fortified tissues were extracted by the method previously described and the purified extracts were assayed to construct the standard curve. Standard curves obtained for each of the tissues are presented in Figure 1.

Assay of Samples. TLC plates were delineated into 11 channels, and each channel was subdivided into three sections for a total of 33 blocks. Triplicate 10-μL aliquots of each sample and of fortified tissue extracts were spotted separately in adjacent blocks. The plate was then placed in a Nunc-bioassay dish and was overlayed with 170 mL of *S. lutea* inoculated medium. The dish was covered, left at room temperature for 30 min, and then incubated overnight at 37 °C. The plate was then sprayed with 5% aqueous 2,3,5-triphenyl-2*H*-tetrazolium chloride solution. This treatment facilitates the observation of clear zones of inhibition on a red background. A sheet of Xerox transparancy was overlayed on the agar bed, and the inhibition zones were traced with a fine tipped pen. For

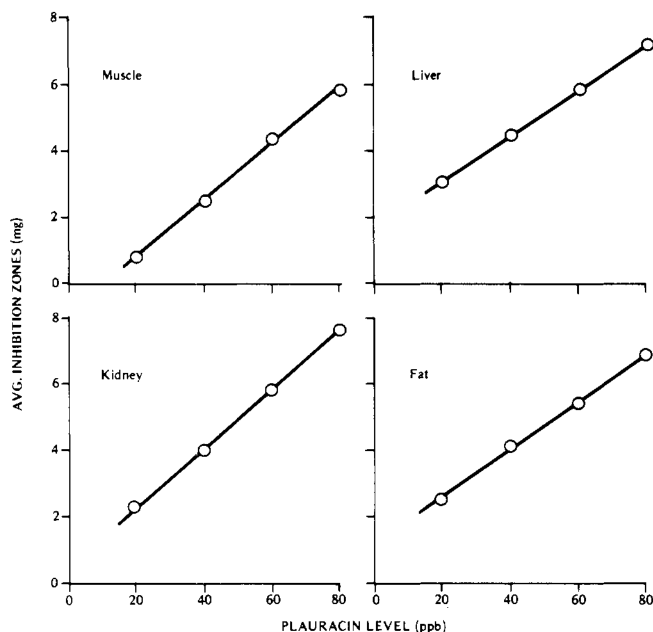

Figure 1. Standard curves employed for assay of plauracin in various swine tissues.

Table I. Percent Recovery of Plauracin from Swine Tissues Fortified at 100 or 50 ppb

Swine tissues	% recov[a]	
	100 ppb	50 ppb
Liver	85 ± 3	86 ± 9
Muscle	96 ± 5	103 ± 7
Kidney	87 ± 4	91 ± 8
Fat	91 ± 5	96 ± 3
Av	90 ± 5	94 ± 7

[a] Average of six replicate samples ± standard deviation.

quantitation, the inhibition zones were measured by the Xerox copying method (Winterlin et al., 1968). In this case the traced zones were copied and each representative zone was cut and weighed on an analytical balance.

RESULTS AND DISCUSSION

Feed and Drug Intake. The average feed consumption was found to be 2.58 kg/pig a day, representing an average daily intake of 3.38 mg of plauracin/kg of body weight. The average weight gain was 19.9 ± 5.3 kg in 29 days.

Tissue Residues. The present assay was found to be linear over the range of 20 to 80 ppb (Figure 1). Higher antibiotic levels could be assayed by diluting the final tissue extract. As seen in Table I, the mean recovery of plauracin from various tissues fortified at 50 and 100 ppb ranged from 85 to 103% with standard deviation varying from ±3 to ±9%. These recovery studies indicate that the components of plauracin are carried through the isolation and purification steps quantitatively and reproducibly.

Liver, muscle, kidney and fat from swine maintained for 29 days on plauracin-medicated feed and slaughtered 0, 1, 2, or 3 days after drug withdrawal were assayed according to the method described. Except for fat, no plauracin levels were detected (<20 ppb) in any of the samples analyzed. Fat contained 25 ppb at zero withdrawal but no detectable levels afterward. It could be concluded that the use of plauracin as a feed additive in swine would result in no detectable antibiotic residues in the major edible tissues if animals were subjected to a 1-day withdrawal period.

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Accumulation and Depletion of Some Organochlorine Pesticides in Broiler Breeder Hens during the Second Laying Cycle

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During the second laying cycle of broiler breeder hens, low levels of organochlorine pesticides (HCB, α-HCH, β-HCH, γ-HCH, heptachlor, p,p'-DDT, and dieldrin) were administered via their food. Accumulation ratios (levels in product/levels in the food) were higher than during the first laying cycle and ranged for fat on a fat basis from 25 for β-HCH to 1.8 for α-HCH. In eggs, on a whole egg basis the range was 2.3 for β-HCH to 0.16 for α-HCH, and on a fat basis the figures were 20 for β-HCH to 1.4 for α-HCH. Giving noncontaminated food during 11 weeks resulted only in a drastic decline of α- and γ-HCH residues in eggs and fat. Correlation coefficients of residues within hens in abdominal, intramuscular, and egg fat were found to be high (>+0.9).

The strong accumulative properties of some organochlorine pesticides in broiler breeder hens have previously been demonstrated (Kan and Tuinstra, 1976b). The accumulation ratios calculated from that experiment were well in line with literature data, although relatively high figures were found. One of the main factors influencing accumulation—egg production—has been clearly illustrated by Cecil et al. (1973). After feeding a low calcium diet, which resulted in 60% egg production as compared to 90% in the control group, they showed that residues of

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