LITERATURE CITED

AOAC. *Official Methods of Analysis*, 14th ed.; Association of Official Analytical Chemists: Washington, DC, 1984.

Association of the German Fruit Juice Industry. *RSK Values, The Complete Manual.* Flüssiges Obst GmbH: Bonn, Federal Republic of Germany, 1987.

Brause, A. R.; Raterman, J. M.; Petrus, D. R.; Doner, L. W. Verification of Authenticity of Orange Juice. *J. Assoc. Off. Anal. Chem.* **1984**, *67*, 535.

Chapman, H. D.; Pratt, P. F. Flame phomtometric procedures for calcium, magnesium, sodium, and potassium. In *Methods of Analysis for Soils, Plants, and Water*; University of California: Berkeley, 1961.

Dekker, R. F. De-bittering of citrus fruit juices: specific removal of limonin and other bitter principles. *Aust. J. Biochem.* **1988**, *2*, 65–76.

Guadagni, D. G.; Maier, V. P.; Turnbaugh, J. G. Effects of some citrus juice constituents on taste thresholds for limonin and naringin bitterness. *J. Sci. Food Agric.* **1973**, *24*, 1277–1288.

Johnson, R. L.; Chandler, B. V. Debittering and de-acidification of fruit juices. *Food Tech. Aust.* **1986**, *38*, 294–297.

Kimball, D. A.; Norman, S. I. Changes in California navel juice during commercial debittering. *J. Food Sci.* **1990**, *55*, 273–274.

Konno, A.; Misaki, M.; Toda, J.; Wada, T.; Yasumatsu, K. Bitterness reduction of naringin and limonin by β-cyclodextrin. *Agric. Biol. Chem.* **1982**, *46*, 2203–2208.

Kuneman, D. W.; Braddock, J. K.; McChesney, L. L. HPLC profile of amino acids in fruit juices as their (1-fluoro-2,4-dinitrophenyl)-5-L-alanine amide derivatives. *J. Agric. Food Chem.* **1988**, *36*, 6–9.

Maeda, H.; Takahashi, Y.; Miyake, M.; Ifuku, Y. Studies on the quality improvement of citrus juices and utilization of peels with ion exchange resins. 1. Removal of bitterness and reduction of acidity in Hassaku (C. Hassaku Hort. Ex Tanaka) juice with ion exchange resins and adsorbents. *Nippon Shokuhin Kogyo Gakkaishi* **1984**, *31*, 413–420.

Maier, V. P.; Beverly, G. D. Limonin monolactone, the nonbitter precursor responsible for delayed bitterness in certain citrus juices. *J. Food Sci.* **1968**, *33*, 488–492.

Marcy, J. E.; Hansen, A. P.; Graumlich, T. R. Effect of storage temperature on the stability of aseptically packaged concentrated orange juice and concentrated orange drink. *J. Food Sci.* **1989**, *54*, 227–230.

Nisperos, M. O.; Robertson, G. L. Removal of naringin and limonin from grapefruit juice using polyvinylpyrrolidone. *Philipp. Agric.* **1982**, *65*, 275–282.

Praschan, V. C. In *Quality Control Manual for Citrus Processing Plants*; Intercit: Safety Harbor, FL, 1976.

Shaw, P. E.; Buslig, B. S. Selective removal of bitter compounds from grapefruit juice and from aqueous solution with cyclocyclodextrin polymers and with Amberlite XAD-4. *J. Agric. Food Chem.* **1986**, *34*, 837–840.

Stutsman, M. J. Overview of Compliance and Regulatory Issues. Presented at the Fruit Juice Adulteration Workshop; General Physics Corp.: Herndon, VA, 1988.

U.S. Government. Code of Federal Regulations, Title 21 146.146, 146.153, 1964.

U.S. Government. Code of Federal Regulations, Title 21 101.9, 1988.

Received for review September 25, 1989. Accepted February 21, 1990.

Registry No. Limonin, 1180-71-8; vitamin C, 50-81-7; sodium, 7440-23-5; potassium, 7440-09-7; copper, 7440-50-8; calcium, 7440-70-2.

Limonoid Model Insect Antifeedants

Michael D. Bentley,*,† Mohamed S. Rajab,† Michael J. Mendel,‡ and A. Randall Alford‡

Department of Chemistry and Department of Entomology, University of Maine, Orono, Maine 04469

Two model insect antifeedants, 1α-(3′-furyl)-4β,4aβ-epoxy-5β,8aα-dimethyl-3-oxooctahydro-1*H*-2-benzopyran (IV) and 1β-(3′-furyl)-4α,4aα-epoxy-5β,8aα-dimethyl-3-oxooctahydro-1*H*-2-benzopyran (IX), based on the C and D rings of the citrus limonoid limonin have been prepared. Both were shown to have activity comparable to that of limonin as antifeedants against larvae of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), in no-choice laboratory assays.

The coevolution of insects and plants has resulted in a range of plant defense strategies with an associated array of phytochemical weapons affecting the growth and survival of herbivorous insects (Ehrlich and Raven, 1964). Prominent among these natural products are the insect antifeedants, or feeding deterrents, which depress or terminate insect feeding through mechanisms that may involve chemosensory-based food rejection, toxicity effects, or a combination of modes. Since the expression of these effects may involve starvation of insects and suppression of growth, development, and reproduction, application of antifeedants as components of integrated pest management programs offers considerable promise. Other potential advantages not shared by many conventional insecticides may include high pest target specificity and facile biodegradation. On the other hand, many of the most potent insect antifeedants thus far isolated are of high structural complexity, and total synthesis of these compounds for agricultural applications will not likely be economically feasible (Menn, 1983). As an example, one of the most prominent antifeedants is azadirachtin

† Department of Chemistry.
‡ Department of Entomology.

0021-8561/90/1438-1400$02.50/0

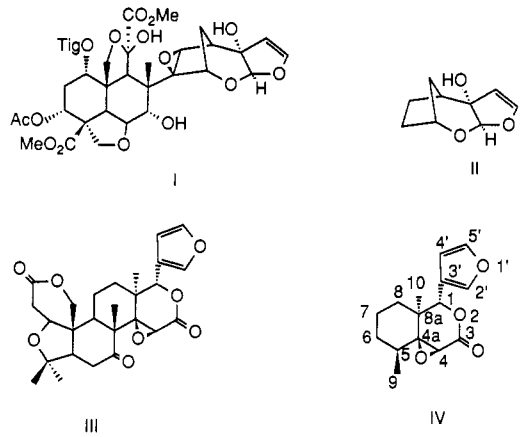

Figure 1.

(I) (Figure 1), isolated from the seeds of the Indian neem tree, *Azadirachta indica* (A. Juss) (Bilton et al., 1987; Broughton et al., 1986; Butterworth and Morgan, 1968; Kraus et al., 1985; Nakanishi, 1975). This limonoid displays antifeedant activity against more than 60 insect species (Warthen, 1979), but its high structural complexity has thus far precluded synthesis, and its application is limited to use of crude extracts of the neem seeds. In the area of medicinal chemistry, considerable success has been derived from synthesis of model compounds based on the structures of biologically active natural products, and in the agricultural chemical area, synthetic pyrethroids and insect juvenile hormone mimics have proven to be highly effective. Ley et al. (1987) have reported the synthesis of the first model antifeedant (II) based on the structure of azadirachtin. In a recent report from our laboratory, the results of structure–activity studies on limonin (III), a citrus limonoid active as an antifeedant against the Colorado potato beetle, *Leptinotarsa decemlineata*, were described (Bentley et al., 1988). The presence of the furan and epoxide functions was found to be critical for activity, a fact that paved the way for the rational design of an antifeedant for this insect. In the present paper, we describe the synthesis of two limonin-based model antifeedants and demonstrate their activity against *L. decemlineata* larvae.

MATERIALS AND METHODS

NMR spectra were obtained with a Varian XL-200 system (δ, J values in hertz). The ^{1}H NMR NOESY spectrum was obtained on a 500-MHz GE NMR spectrometer at the University of Texas by Dr. Ben Shoulders. Medium-resolution mass spectra were measured with a HP-5985-B GC–MS system and high-resolution mass spectra with a VG-70E by Dr. George Goodloe at Auburn University. Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected.

Compounds VIa and VIb. To a solution of 34.1 mmol of 3-lithiofuran in THF at −78 °C [prepared from 3-bromofuran (5 g, 34.1 mmol) and *n*-butyllithium (34.1 mmol) in heptane] was added a solution of V (3.35 g, 17.05 mmol) (Fukuyama and Tokoroyama, 1973) over 15 min. After the mixture was stirred at this temperature for 15 min and at room temperature for an additional 48 h, water was added to the mixture and the resulting mixture extracted with 3 × 100 mL of $CHCl_3$. The combined extracts were dried over Na_2SO_4 and concentrated under vacuum to yield a 1:1 mixture (^{1}H NMR) of epimers VIa and VIb. Pure VIa was obtained after four successive recrystallizations from acetone–hexane. Pure VIb was obtained by concentration of the combined mother liquors and silica gel column chromatography eluted with 20% acetone–hexane. VIa was obtained as white needles (mp 130–132 °C) from acetone–hexane: ^{1}H NMR (200 MHz, $CDCl_3$, TMS) δ 107 (3 H, s, 10-Me),

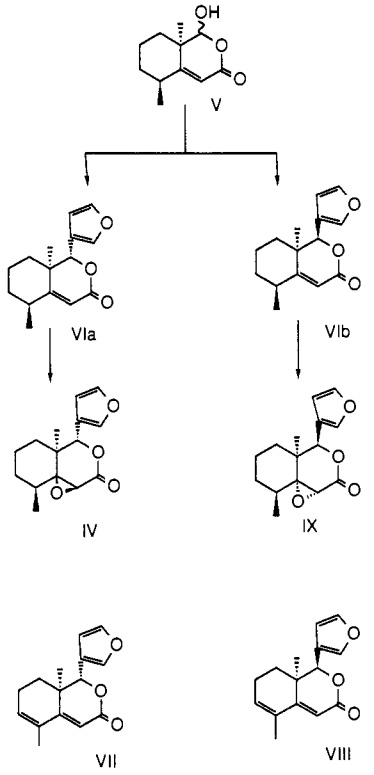

Figure 2.

Table I. Activity of Limonin and Model Compounds as Antifeedants in No-Choice Assays against *L. decemlineata* Larvae

dosage, μg/cm^2	% feeding redn (±SE)		
	IV[a]	IX[b]	limonin[c]
100	100 (8.8)	88.8 (6.7)	81.5 (2.8)
31.7	82.9 (3.2)	84.7 (6.7)	71.8 (8.0)
10	71.0 (6.4)	83.3 (3.2)	56.9 (7.9)
3.17	30.0 (8.0)	9.8 (6.7)	30.4 (4.1)
1	16.9 (5.3)	0.0 (10.1)	7.5 (3.2)

[a] ED_{50} = 6.5 μg/disk (0.025 μmol/disk). [b] ED_{50} = 7.0 μg/disk (0.027 μmol/disk). [c] ED_{50} = 8.3 μg/disk (0.0177 μmol/disk).

1.17 (3 H, d, J = 3.7, 9-Me), 1.20–2.00 (6 H, m, H-6, H-7, H-8), 2.40 (1 H, m, H-5), 5.05 (1 H, s, H-1), 5.86 (1 H, m, H-4), 6.44 (1 H, m, H-4′), 7.42 (1 H, m, H-5′), 7.46 (1 H, m, H-2′); ^{13}C NMR (50.3 MHz, $CDCl_3$, TMS) δ 17.72 (C-10, C-9), 20.84, 34.50, 35.63 (C-6, C-7, C-8), 33.56 (C-5), 39.27 (C-8a), 81.42 (C-1), 110.02 (C-4′), 112.21 (C-4), 120.19 (C-3′), 140.89 (C-5′), 142.66 (C-2′), 165.28 (C-4a), 171.05 (C-3); HRMS for $C_{15}H_{18}O_3$, calcd 246.1251, found 246.1257. Epimer VIb was also obtained as white needles (mp 100–102 °C) from acetone–hexane: ^{1}H NMR ($CDCl_3$) δ 1.17 (3 H, d, J = 6.4, 9-Me), 1.32 (3 H, s, 10-Me), 1.40–2.00 (6 H, m, H-6, H-7, H-8), 2.55 (1 H, m, H-5), 5.08 (1 H, s, H-1), 5.88 (1 H, m, H-4), 6.36 (1 H, m, H-4′), 7.37 (1 H, m, H-5′), 7.42 (1 H, m, H-2′); ^{13}C NMR ($CDCl_3$) δ 17.39, 23.56 (C-10, C-9), 20.92, 33.84, 36.31 (C-6, C-7, C-8), 33.84 (C-5), 39.92 (C-8a), 80.68 (C-1), 109.86 (C-4′), 111.51 (C-4), 121.56 (C-3′), 141.46 (C-5′), 143.00 (C-2′), 164.50 (C-4a), 170.36 (C-3); HRMS for $C_{15}H_{18}O_3$, calcd 246.1251, found 246.1257.

Compounds IV and IX. To 500 mg of VIa in 20 mL of MeOH was added 2 mL of 50% H_2O_2 at 0 °C. One milliliter of 6 N NaOH was then added dropwise over 10 min and stirring continued at room temperature for 48 h. The mixture was then poured into water and extracted with 3 × 50 mL portions of $CHCl_3$. The combined extracts were dried over Na_2SO_4 and concentrated under vacuum to yield IV (0.43 g, 80%) as a pale yellow solid that was recrystallized from acetone–hexane to give colorless needles: mp 141–143 °C; ^{1}H NMR ($CDCl_3$) δ 0.78 (3 H, d, J = 6.7, 9-Me), 1.03 (3 H, s, 10-Me), 1.20–1.80 (6 H, m, H-6, H-7, H-8), 2.30 (1 H, m, H-5), 3.66 (1 H, s, H-4), 5.46 (1 H, s, H-1), 6.36 (1 H, m, H-4′), 7.39 (2 H, m, H-5′, H-2′); ^{13}C NMR

δ 13.58, 14.42 (C-10, C-9), 20.73 (C-7), 29.42 (C-5), 32.61 (C-6, C-8), 38.63 (C-8a), 52.92 (C-4), 68.10 (C-4a), 77.82 (C-1), 110.03 (C-4′), 120.26 (C-3′), 140.98 (C-5′), 142.90 (C-2′), 167.98 (C-3); HRMS for $C_{15}H_{18}O_4$, calcd 262.1200, found 262.1206. In a similar manner, IX was prepared from VIb in 80% yield as colorless needles: mp 114–115 °C; ^{1}H NMR ($CDCl_3$) δ 0.78 (3 H, d, J = 6.7, 9-Me), 1.12 (3 H, s, 10-Me), 1.40–2.00 (6 H, m, H-6, H-7, H-8), 2.40 (1 H, m, H-5), 3.59 (1 H, s, H-4), 5.35 (1 H, s, H-1), 6.37 (1 H, m, H-4′), 7.40 (2 H, m, H-5′, H-2′); ^{13}C NMR ($CDCl_3$) δ 13.40, 17.29 (C-10, C-9), 20.46 (C-8), 29.17, 34.70 (C-6, C-7), 30.09 (C-5), 39.29 (C-8a), 50.94 (C-4), 69.43 (C-4a), 76.63 (C-1), 110.10 (C-4′), 120.14 (C-3′), 141.05 (C-5′), 142.76 (C-2′), 167.82 (C-3); HRMS for $C_{15}H_{18}O_4$, calcd 262.1200, found 262.1204.

***dl*-Pyroangolensolide (VII).** VIa (100 mg) and NBS (80 mg) were reluxed for 45 min in 20 mL of CCl_4. Water was added, and the mixture was extracted several times with $CHCl_3$. The combined extracts were evaporated, and the residue was treated with excess K_2CO_3 in MeOH to yield VII: mp 144–146 °C (lit. mp 146.2–146.8 °C (Tokoroyama et al., 1988)); ^{1}H NMR ($CDCl_3$) δ 1.04 (3 H, s), 1.88 (3 H, d, J = 2), 2.28 (2 H, m), 5.14 (1 H, s), 5.88 (1 H, m), 6.20 (1 H, m), 6.50 (1 H, m), 7.52 (2 H, m). This spectrum agreed closely with that reported in the literature (Tokoroyama et al., 1988).

***dl*-Epipyroangolensolide (VIII).** VIII (mp 138–140 °C (lit. mp 140–141.3 °C (Tokoroyama et al., 1988)) was prepared from VIb by the same procedure as described above for VII: ^{1}H NMR ($CDCl_3$) δ 1.38 (3 H, s), 1.90 (3 H, d, J = 2), 2.30 (2 H, m), 5.14 (1 H, s), 5.94 (1 H, s), 6.18 (1 H, m), 6.32 (1 H, m), 7.44 (2 H, m). This spectrum agreed closely with that reported in the literature (Tokoroyama et al., 1988).

BIOASSAYS

Insect-rearing procedures were modifications of those described by DeWilde (1957) and Boiteau and Alford (1983) using potato foliage (*Solanum tuberosum* L., cv. Katahdin) grown in a greenhouse. All beetle life stages were kept in environmental chambers at 28 °C and a 18:6 (L:D) photoperiod. Fourth instars were used for all tests. The assays were conducted as described by Alford et al. (1987) in arenas constructed from plastic Petri dishes (15 × 90 mm). A circle of moistened filter paper (9-cm diameter) was placed on the floor of each arena. Potato foliage disks (11-mm diameter) were cut with a cork borer from leaves with well-developed primary leaflets. The disks were anchored to the filter paper by small map pins. Treated leaf disks were coated on the upper surface with 50 μL of a solution of the test compounds in acetone; control leaf disks received 50 μL of acetone alone. Acetone was allowed to evaporate before assays were initiated. For these no-choice assays (Jermy et al., 1968), 10 treated and 10 untreated control disks were run for each test and each test was replicated on 3 separate days. At the beginning of each test, two fourth instars (within 24 h of molt) were placed in each arena for 6–8 h; assays began 4–5 h after the start of the photophase. Arenas were placed in clear plastic ventilated crisper boxes containing moist paper toweling and placed in an environmental chamber at 28 °C. Consumption was determined by weighing the oven-dried (24 h at 110 °C) remains of disks for each arena. Initial weights were determined for the tests by drying 100 additional disks; mean daily weights of these blank disks were assumed to be the initial weights of the assay disks. Calculations of amounts of disks eaten were made by subtracting the weight of the remains from the initial weight for the appropriate test. Percentage of feeding reduction was calculated for each arena by the equation (Alford et al., 1987) % FR = [1 − treatment consumption/control consumption] × 100.

RESULTS AND DISCUSSION

Our antifeedant structure–activity studies on limonin led us to the conclusion that the groups essential for the activity of that molecule were the epoxide and the furan, and this guided us in developing a likely candidate for a model antifeedant. We chose IV (Figure 1) as a logical target compound, which also bore the advantage of the excellent earlier synthetic work of Fukuyama and Tokoroyama (1973), leading to the synthesis of the lactol precursor V (Figure 2). Compounds IV and IX were synthesized according to the scheme presented in Figure 2. The epimeric mixture of lactols V was prepared in six steps from 2,6-dimethylcyclohexanone in 46% overall yield according to the procedures of Fukuyama and Tokoroyama (1973). Reaction of V with 3-furyllithium at −78 °C led to a 1:1 mixture of VIa and VIb, which was readily separated by recrystallization. The stereochemistries of VIa and VIb were confirmed by conversion to the known pyroangolensolide (VII) and epipyroangolensolide (VIII), respectively. Epoxidation of VIa with 50% H_2O_2 in base led to the diastereofacially selective formation of the target compound IV, and in a similar manner, epoxide IX was prepared from VIb. The stereochemistries of IV and IX were differentiated by examination of the NOESY ^{1}H NMR spectrum of IX, which exhibited correlations of H-1α with H-10, H-10 with H-5α, H-5α with H-9, and H-9 with H-4.

Both compounds IV and IX were evaluated as antifeedants in no-choice assays against Colorado potato beetle larvae. The results are summarized in Table I with the results of earlier, similar assays for limonin (Bentley et al., 1988) also included for comparison. Both IV and IX exhibited substantial levels of activity comparable to that found for limonin. It is quite interesting that a very simplified limonoid model such as IV, differing significantly from limonin in both polarity and stereochemistry, approximates the activity of the complex natural product. With its considerable economy in functionality, IV is much less polar than limonin. In addition, IV was assayed as a racemic mixture, while limonin was evaluated as the naturally occurring enantiomer. A further stereochemical difference lies in the conformation of the limonin C-ring and the corresponding ring in IV; in the former, ring C is locked in the boat conformation, while in the latter, the chair is preferred. The apparent lack of rigid stereochemical requirements for the observed activity is also reflected in the similar observed activities for isomers IV and IX. These results imply that the biological response is not triggered by a highly stereospecific interaction between the antifeedant and a reactor protein. We have conducted no mode of action studies on IV and are not certain at this point whether its mode is identical with that of limonin. Our preliminary investigation of the mode of action of limonin, however, indicates that chemoreception is probably not the primary mode of action in the food ingestion decision in these assays and that the observed depression in feeding rate induced by limonin (Alford et al., 1987) may be due to a toxicity effect taking place after ingestion. The lack of highly specific structural requirements for this antifeedant activity lends promise to the possibility of a reasonably simple and practical synthetic antifeedant against *L. decemlineata*. We are pursuing this goal with several related systems.

ACKNOWLEDGMENT

We thank Dr. Ben Shoulders of the University of Texas for NOESY spectra. This project was supported in part by the Maine Agricultural Experiment Station and the NE-154 Regional Research Project, Integrated Pest Management for Potatoes (Grant No. 86-CSRS-2-2759). This paper is published as MAES No. 1430.

LITERATURE CITED

Alford, A. R.; Cullen, J. A.; Storch, R. H.; Bentley, M. D. Antifeedant Activity of Limonin against the Colorado Potato Beetle. *J. Econ. Entomol.* **1987**, *80*, 575–578.

Bentley, M. D.; Rajab, M. S.; Alford, A. R.; Mendel, M. J.; Hassanali, A. Structure-Activity Studies of Modified Citrus Limonoids as Antifeedants for Colorado Potato Beetle Larvae, *Leptinotarsa decemlineata*. *Entomol. Exp. Appl.* **1988**, *49*, 189–193.

Bilton, J. N.; Broughton, H. B.; Jones, P. S.; Ley, S. V.; Lidert, Z.; Morgan, E. D.; Rzepa, H. S.; Sheppard, R. N.; Slawin, A. M. Z.; Williams, D. J. An X-Ray Crystallographic, Mass Spectrometric, and NMR Study of the Limonoid Insect Antifeedant Azadirachtin and Related Derivatives. *Tetrahedron* **1987**, *43*, 2805–2815.

Boiteau, G.; Alford, A. Synchronization of Colorado Potato Beetle (Coleoptera: Chrysomelidae) Emergence by Temporary Storage of Eggs at Low Temperature. *Can. Entomol.* **1983**, *115*, 1233–1234.

Broughton, H. B.; Ley, S. V.; Slawin, A. M. Z.; Williams, D. J.; Morgan, E. D. X-Ray Crystallographic Structure Determination of Detigloyldihydroazadirachtin and Reassignment of the Structure of the Limonoid Insect Antifeedant Azadirachtin. *J. Chem. Soc., Chem. Commun.* **1986**, 46–47.

Butterworth, J. H.; Morgan, E. D. Isolation of a Substance that Suppresses Feeding in Locusts. *Chem. Commun.* **1968**, 23–24.

DeWilde, J. Breeding and Rearing Colorado Potato Beetle under Controlled Conditions. *Z. Pflanzenkr. Pflanzenpathol. Pflanzenschutz.* **1957**, *64*, 589–593.

Ehrlich, P. R.; Raven, A. H. Evolution Butterflies and Plants: A Study in Coevolution. **1964**, *18*, 586–608.

Fukuyama, Y.; Tokoroyama, T. Total Synthesis of Pyroangolensolide. *Tetrahedron Lett.* **1973**, 4869–4872.

Jermy, T.; Hanson, F. E.; Dethier, V. G. Induction of Specific Food Preferences in Lepidopterous Larvae. *Entomol. Exp. Appl.* **1968**, *11*, 211–230.

Kraus, W.; Bokel, M.; Klenk, A.; Pohnl, H. The Structure of Azadirachtin and 22,23-Dehydro-23β-methoxyazadirachtin. *Tetrahedron Lett.* **1985**, *26*, 6435–6438.

Ley, S. V.; Santafianos, D.; Blaney, W. M.; Simmonds, M. S. J. Synthesis of a Hydroxy Dihydrofuran Acetal related to Azadirachtin: A Potent Insect Antifeedant. *Tetrahedron Lett.* **1987**, 221–224.

Menn, J. J. Present Insecticides and Approaches to Discovery of Environmentally Acceptable Chemicals for Pest Management. In *Natural Products for Innovative Pest Management*; Whitehead, D. L., Bowers, W. S., Eds.; Pergamon: Oxford, 1983; pp 5–31.

Nakanishi, K. Structure of the Insect Antifeedant Azadirachtin. *Recent Adv. Phytochem.* **1975**, *9*, 283–298.

Tokoroyama, T.; Fukuyama, Y.; Kotsuji, Y. Synthetic Studies on Terpenoid Compounds. Part 14. Total Synthesis of Pyroangolensolide. *J. Chem. Soc., Perkin Trans.* **1988**, 445–449.

Warthen, J. D. *Azadirachta indica: A Source of Insect Feeding Inhibitors and Growth Regulators*; Agriculture Review and Manuals, USDA-SEA ARM-NE-4; U.S. GPO: Washington, DC, 1979; 21 pp.

Received for review October 2, 1989. Accepted January 17, 1990.

Residues of Dichlorvos in Atlantic Salmon (*Salmo salar*) after Delousing

Tor Einar Horsberg* and Tonje Høy

Department of Pharmacology and Toxicology, The Norwegian College of Veterinary Medicine, P.O. Box 8146 Dep, N-0033 Oslo 1, Norway

Oddvar Ringstad

Center for Industrial Research, P.O. Box 124 Blindern, N-0314 Oslo 3, Norway

Liver and muscle tissues from Atlantic salmon (*Salmo salar*) were analyzed for residues of dichlorvos after treatment against salmon lice infestations at water temperatures of 4 and 12 °C. Dichlorvos was extracted from tissue samples with cyclohexane and acetone, cleaned by gel permeation chromatography, and determined by gas chromatography (glass capillary column and alkali flame nitrogen–phosphorus specific detector). Though at a water temperature of 4 °C residues were detectable in muscular tissue for up to 3 days after treatment, at 12 °C they could only be detected in samples taken directly after treatment. In liver tissue, residues were detectable for up to 3 days after treatment at 4 °C, while at 12 °C traces of dichlorvos were evident in some samples for up to 6 days after treatment.

The ectoparasitic copepod *Lepeophtheirus salmonis*, commonly known as the salmon louse, causes severe, annually recurring disease problems in farmed salmonids in Norway.

Bath treatment with the organophosphorus compounds trichlorfon [metrifonate, *O,O*-dimethyl (1-hydroxy-2,2,2-trichloroethyl)phosphonate] or dichlorvos [DDVP, *O,O*-dimethyl *O*-(2,2-dichlorovinyl) phosphate], has been employed to control this pest. The structures of these organophosphates are shown in Figure 1. Both substances act by lethal inhibition of cholinesterases in the parasite. Residue studies have shown that trichlorfon is

0021-8561/90/1438-1403$02.50/0