Isolation and Structure Elucidation of Growth Inhibitors for Silkworm Larvae from Avocado Leaves

Ching-Fun Chang, Akira Isogai,\*<sup>1</sup> Toshiya Kamikado,\*<sup>2</sup> Shigeo Murakoshi,\*<sup>3</sup> Akira Sakurai and Saburo Tamura

The Institute of Physical and Chemical Research, Wako-shi, Saitama-ken \*1Department of Agricultural Chemistry, The University of Tokyo, Bunkyo-ku, Tokyo \*2Kanagawa Prefectural Institute for Sericultural Science, Ebina-shi, Kanagawa-ken Received February 26, 1975

During our screening project to find substances biologically active to insects,<sup>1,2)</sup> we noticed that fresh leaves of avocado (*Persea americana* Mill.) inhibited the growth of silkworm larvae (*Bombyx mori* L.) by oral administration. Then we attempted the isolation of the active principle(s) in the plant and successfully obtained two components. In this paper we wish to report preliminarily our experimental result leading to structure elucidation of the compounds.

Fresh avocado leaves (3.1 kg) grown in a greenhous at the herbary of Takeda Chemical Industries, Ltd. in Kyoto were harvested at spring of 1974 and were extracted with methanol. The extract was fractionated by the conventional method into ethyl acetate-soluble acidic, neutral and basic fractions, of which the neutral one revealed biological effect. The active fraction was applied to silicic acidcelite column chromatography using benzene and benzene-ethyl acetate. An eluate with benzene containing 5% ethyl acetate was successively applied to florisil column chromatography using the same solvent system and silicic acid-celite column chromatography. Thus, one active component (I) was obtained as crystals (120 mg), mp  $121 \sim 122^{\circ}$ C, and another (II<sub>A</sub>) as an oil (1.8 g) after preparative thin-layer chromatography using silicic acid GF<sub>254</sub> and chloroform-methanol (97: 3).

I causes direct pupation in the fourth instar larvae of silkworm without the fourth molting at the concentration of 100 ppm in the diet, while II<sub>A</sub> acts toxic to the larvae at 200 ppm.



I,  $C_{22}H_{28}O_6$ , was identified as dimethyl sciadinonate<sup>3)</sup> by comparing its physicochemical and spectrometric data with those of an authentic specimen kindly supplied by Professor M. Ishikawa of Tokyo Medical and Dental University.

 $II_{A}$ ,  $[\alpha]_{D}^{24}$ +11.3 (c=4.5 in CHCl<sub>3</sub>), was positive to 2,4-dinitrophenylhydrazine. The molecular formula of  $\Pi_A$  was assigned as  $C_{23}H_{40}O_4$ by high resolution mass spectrometry (M-60, m/e 320.2793; calcd. for C<sub>21</sub>H<sub>36</sub>O<sub>2</sub>, 320.2714) as well as by elemental analysis of its tetrahydro derivative (II<sub>B</sub>),  $C_{23}H_{44}O_4$ , M<sup>+</sup> 384, which was obtained by catalytic hydrogenation of  $II_A$  on  $PtO_2$  in methanol. IR of  $II_A \nu_{max}^{Film}$ cm<sup>-1</sup>: 3300 (hydroxyl), 1735 (ester carbonyl), 1710 (isolated ketone), 1620 (double bond). NMR  $\delta_{TMS}^{CDC1_3}$ : 0.88 (3H, t, J=6.0 Hz, -(CH<sub>2</sub>)<sub>n</sub>-CH<sub>3</sub>), 1.26 (16H, broad s), 1.8~2.2 (4H, m, -CH<sub>2</sub>-CH=CH-), 2.09 (3H, s, CH<sub>3</sub>COO-), 2.78 (2H, broad t, -CH=CH-CH2-CH=CH-), 2.45 (2H, t, J=7.5 Hz), 5.36 (4H, m, -CH= CH-). In the NMR spectrum of  $II_{B}$ , signals due to olefinic and allylic protons were unobservable while strong signals of methylene protons (30 H) were detected. Signals at  $\delta$  4.30 (1H, m), 4.04~4.20 (2H, m) and 2.62 (2H, d, J=6.0 Hz) in II<sub>A</sub> shifted to  $\delta$  5.42 (1H, m), 4.10 (1H, dd, J=12.0 and 5.0 Hz), 4.28 (1H, dd, J=12.0 and 4.0 Hz) and 2.70 (2H, m) in II<sub>C</sub>,  $C_{25}H_{46}O_5$ , M<sup>+</sup> 426, obtained by

<sup>\*3</sup> Present address: Research Laboratories, Agricultural Chemicals Division, Takeda Chemical Industries, Ltd., Yodogawa-ku, Osaka.

acetylation of  $II_B$  with acetic anhydridepyridine. On the basis of the spectral data thus far mentioned, the structure of  $II_A$  was deduced to be CH<sub>3</sub>COOCH<sub>2</sub>-CH(OH)-CH<sub>2</sub>-CO-CH<sub>2</sub>-C<sub>16</sub>H<sub>29</sub>.

Treatment of II<sub>A</sub> with *p*-toluenesulfonic acid, followed by preparative thin-layer chromatography using silicic acid GF<sub>254</sub> and hexane, afforded a new compound (II<sub>D</sub>), C<sub>21</sub>H<sub>34</sub>O, M<sup>+</sup> 302. In the NMR spectrum of II<sub>D</sub>, signals corresponding to 2-substituted furan were ovserved at  $\delta$  7.26 (1H, m), 6.25 (1H, dd, *J*=2 and 3 Hz) and 5.98 (1H, dd, *J*=1 and 3 Hz). Formation of the furan ring vigorously supports the structural assignment for the oxygenated portion in II<sub>A</sub>.<sup>4</sup>)

Ozonolysis of  $II_{D}$  followed by oxidative decomposition yielded caproic acid, azelaic acid and azelaic half-aldehyde, which were identified as the forms of methyl esters with GC-MS, respectively. This indicates the locations of double bonds in the  $C_{16}H_{29}$ -portion.

Thus, the structure of  $II_A$  was established as 1-acetoxy-2-hydroxy-4-oxo-heneicosa-12, 15diene. The configuration of the double bonds in  $II_A$  is considered to be *cis*, because the IR

$$\begin{array}{c} \mathsf{CH}_2-\mathsf{CH}-\mathsf{CH}_2-\ddot{\mathsf{C}}-\mathsf{CH}_2-\mathsf{R}_2\\ \mathsf{oAc}\ \mathsf{oR}_1\\ \\ \mathsf{R}_1\\ \mathsf{R}_1\\ \mathsf{R}_2\\ \mathsf{II}_A\\ \mathsf{H}\\ \mathsf{H}\\ \mathsf{(CH}_2)_6-\mathsf{CH}=\mathsf{CH}-\mathsf{CH}_2-\mathsf{CH}=\mathsf{CH}-\mathsf{(CH}_2)_4-\mathsf{CH}_3\\ \\ \mathsf{II}_B\\ \mathsf{H}\\ \mathsf{(CH}_2)_{15}-\mathsf{CH}_3\\ \\ \mathsf{II}_C\\ \mathsf{Ac}\\ \mathsf{(CH}_2)_{15}-\mathsf{CH}_3\\ \end{array}$$

Ö

spectrum of the compound reveals no absorption band around 960 cm<sup>-1</sup> which is characteristic of *trans* double bond.

Biological activities of I and  $II_A$  will be reported in the near future together with details of structure elucidation of  $II_A$ .

## REFERENCES

- A. Isogai, C.-F. Chang, S. Murakoshi, A. Suzuki and S. Tamura, *Nippon Nôgeikagaku Kaishi*, 47, 443 (1973).
- T. Kamikado, C.-F. Chang, S. Murakoshi and S. Tamura, Agr. Biol. Chem., 39, 833 (1975).
- C. Kaneko, T. Tsuchiya and M. Ishikawa, *Chem. Pharm. Bull.*, 11, 271, 1346 (1963).
- Y. Kashman, I. Néeman and A. Lifshitz, *Tetra*hedron, 25, 4617 (1969).