Synthesis of 18-Octadecanolide, a Major Constituent of Dufour's Gland Secretion of *Colletes* Bees

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18-Octadecanolide, a major constituent of Dufour's gland secretion of *Colletes* bees, was synthesized by polymerization followed by depolymerization of 18-hydroxyoctadecanoic acid which in turn was obtained by hydrogenation of kamlolenic acid (18-hydroxy, 9-*cis*, 11-*trans*, 13-*trans*-octadecatrienoic acid) isolated from *Mallotus philippinensis* seed oil. The structures of the intermediates as well as the final lactone were established by infrared, proton nuclear magnetic resonance and mass spectrometric methods.

Some recent investigations (1-4) have established the occurrence of 18-octadecanolide as a major constituent of Dufour's gland secretions of *Colletes* bees. The lactone appears to participate in the construction of the protective membrane of brood cells of the bees and in recognition of their nests (5,6). The structure of the isolated lactone was established by comparison with 15-penta-(1) and 16-hexadecanolides (2), but not with 18-octadecanolide. The synthesis of the latter by various cumbersome procedures has been reported (7-9). In this work the lactone was prepared by a simple route from 18-hydroxystearic acid prepared from kamla (*Mallotus philippinensis*) seed oil.

MATERIALS AND METHODS

Infrared (IR) spectra were obtained with a Perkin-Elmer Model 283 B spectrophotometer using either KBr wafers or 10% solution in CCl_4. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded in $CDCl_3$ with a JEOL FX 90 Q Fourier Transform NMR spectrometer. Chemical shifts were measured in ppm downfield from the internal standard, tetramethylsilane ($\delta = 0$). Mass spectra were measured with a V.G. Micromass 7070 H mass spectrometer at 70 eV.

Glass plates were coated with a 0.8-mm layer of silica gel G (ACME Synthetic Chemicals, Bombay, India) for thin layer chromatography (TLC), and benzene was used as the developing solvent. Gas liquid chromatographic (GLC) analysis was carried out using a Hewlett-Packard 5840 A unit equipped with a dual hydrogen flame ionization detector (FID), a data processor, and SE-30 column (0.6 m × 6 mm). The column, detector and injection port were maintained at 200, 300 and 250 C, respectively. The flow rate of nitrogen was 30 ml/min.

Preparation of kamlolenic acid. Kamla (*Mallotus philippinensis*) seed oil was extracted from ground seeds by soaking in diethyl ether in an aspirator bottle overnight. Extraction and all the subsequent operations were carried out at room temperature. After three extractions, the pooled ether solution was dried over anhydrous sodium sulfate and the oil recovered by removing the solvent under low pressure. The oil was saponified by leaving it in contact with alcoholic potassium hydroxide solution for a day. The soap solution was diluted with distilled water, and the unsaponifiable matter was removed by repeated extraction with ether. A fresh lot of ether was added to the soap solution and the fatty acids were liberated in the presence of ether using dilute hydrochloric acid. The mixed fatty acids were obtained by removing the solvent in a rotary evaporator. Petroleum ether (100 ml, 40-60 C) was added to the mixed fatty acids (25 g); the main bulk of kamlolenic acid precipitated. The operation was repeated twice. Crystallization of the crude material from ethyl acetate at −5 to 0 C gave pure kamlolenic acid (10 g); m.p. 79 C (lit. 78-79 C).

Preparation of 18-hydroxyoctadecanoic acid. Kamlolenic acid (5 g) was dissolved in ethyl acetate (50 ml) and hydrogenated at room temperature in a Parr hydrogenator at 45 psig using 10% palladium on carbon (0.5 g) until the absorption of hydrogen ceased. The solution was filtered and concentrated, and the product was repeatedly crystallized from the same solvent at −5 to 0 C to get pure 18-hydroxyoctadecanoic acid in 90% yield; m.p. 99 C (lit. 99-101 C) (Found: C, 72.1; H, 12.1. Calcd. for $C_{18}H_{36}O_3$: C, 72.0; H, 12.0%). The acid was further characterized as its methyl ester by various physical methods. Methyl 18-hydroxyoctadecanoate was prepared by esterification of the acid using boron trifluoride etherate in anhydrous methanol; m.p. 61.4 C (lit. 62.4-62.6 C) (Found: C, 72.28; H, 12.15. Calcd. for $C_{19}H_{38}O_3$: C, 72.61; H, 12.10%). GLC purity: 99.8%, IR (KBr): 3350 (OH stretch), 1740 ($COOCH_3$), 2920, 2845, 1250, 1209, 1170 cm^{-1}. NMR: δ 1.26 (30 H, chain methylene protons), 2.32 (2H, C_2 methylene protons alpha to ester group) and 3.66 (5 H, methyl ester protons, $-COOCH_3$, plus terminal methylene protons having hydroxyl group $-CH_2OH$). MS:m/z 314 (0.8%), 296 (1.7%), 284 (19.1%), 283 (1.7%), 282 (4.2%), 143 (16.6%), and 74 (100%).

Preparation of 18-octadecanolide. 18-Hydroxyoctadecanoic acid (1.0 g) was heated at 200-230 C for three hr and then at 240 C/1 torr for 1.5 hr to yield a polyester. The polyester was depolymerized by further heating for two hr in the presence of magnesium chloride (0.1 g) at 260-270 C/0.2 torr. The distillate was collected, dissolved in ether, washed with sodium carbonate solution and purified by preparative silica gel TLC to get pure 18-octadecanolide in 50% yield; m.p. 40.6 C. (Found: C, 76.42; H, 12.1. Calcd. for $C_{18}H_{34}O_2$: C, 76.59; H, 12.06%). GLC purity: 99.9%. IR ($CHCl_3$): 1732 (macrocyclic lactone) 2930, 2852 cm^{-1}. NMR: δ 1.31 (30H, chain methylene protons), 2.29 (2 H, C_2 methylene protons alpha to lactone carbonyl) and 4.09 (2H, methylene protons attached to oxygen, CH_2-O-). MS:m/z 282 (9.0%), 264 (6.6%), 222 (3.3%), 97 (27.9%), 83 (37.7%), 69 (49.2%), 55 (95.1%) and 41 (100%).

RESULTS AND DISCUSSION

Many of the species of solitary and social bees (Apidae; Hymenoptera) make their nests and brood cells in moist soil. The hygroscopic provision of nectar and pollen is

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protected against water, fungi and various soil organisms by a layer of waterproof secretion applied by the nesting female to the soil surrounding the cell. The secretion from the Dufour's gland of *Colletes* bees, suspected to be the source of the cell lining, was found to contain 18-octadecanolide as a major constituent of macrocyclic lactones (1-4). To make a structural study of such lactones, a comparison with synthetic compounds is useful. Therefore, a simple route for synthesis of 18-octadecanolide was undertaken using 18-hydroxyoctadecanoic acid. This acid was obtained by hydrogenation of kamlolenic acid (18-hydroxy, 9-*cis*, 11-*trans*, 13-*trans*-octadecatrienoic acid) which was isolated from kamla seed oil. The methyl ester of 18-hydroxyoctadecanoic acid, obtained by esterification of the acid using boron trifluoride-methanol, showed IR absorption bands at 3350 cm^{-1} due to hydroxyl stretching and at 1740 cm^{-1} due to C = O stretching of an ester group. The NMR spectrum of the methyl ester showed a singlet at δ 3.66 due to methyl ester protons overlapped by a triplet centered at δ 3.64 due to terminal methylene protons having hydroxyl group, a triplet at δ 2.32 due to methylene protons alpha to ester group and an intense signal at δ 1.26 due to chain methylene protons. The mass spectrum of the methyl ester showed a weak molecular ion at m/z 314 as well as other fragment ions at m/z 296 (loss of water), m/z 284 (loss of CH_2O), m/z 282 (loss of methanol), and fragments at m/z 143, 87 and 74 characteristic of long chain ω-hydroxy acid methyl esters (1). The spectral data confirmed the structure of the ester as methyl 18-hydroxyoctadecanoate.

The hydroxyoctadecanoic acid on heating at 200-230 C yielded a linear polyester which was insoluble in organic solvents (benzene or chloroform), suggesting that it had a very high molecular weight. According to Carothers and Hill (10) and Spanagel and Carothers (11-13), bifunctional esterifications involving unit length greater than seven carbons yielded almost exclusively linear polyesters on heating. When the linear polyesters were heated to 200-250 C, the long chains coupled to form still longer chains. The products, 'super polyesters,' had molecular weights above 10,000.

The polyester, on thermal depolymerization using magnesium chloride (13) as catalyst, yielded the corresponding lactone. The purity of the lactone, isolated by preparative silica gel TLC, was found to be 99.9% as determined by GLC on SE-30 column.

Absence of IR absorption bands (Fig. 1) due to carboxyl and hydroxyl stretching vibrations and appearance of a band at 1732 cm^{-1}, which was shifted from 1705 cm^{-1} (C = 0 str. in carboxylic acid) to 1732 cm^{-1}, indicated the formation of macrocyclic lactone. The NMR spectrum showed a triplet at δ 4.09 due to methylene protons attached to oxygen $-CH_2O$, which were slightly deshielded from the position (δ 3.64) as in 18-hydroxyoctadecanoate, confirmed the formation of lactone. The integration of the signal at δ 2.29 showed two protons due to methylene protons alpha to lactone carbonyl $(\text{-}\overset{\overset{\text{O}}{\|}}{\text{C}}\text{-O-})$ and 30 protons at δ 1.31 due to chain methylene protons.

The mass spectrum of the lactone synthesized in the present investigation is the same as that reported for the isolated lactone (2,3). The spectrum showed a distinct molecular ion at m/z 282 indicating the expected molecular weight of the lactone. The other intense fragments in the higher mass end were fragments 264 and 222. The ion at m/z 264 (M-18) indicated that the ring was readily broken in the excited parent ion, thus permitting a transfer of hydrogens from hydrocarbon portions to the oxygen and resulting in the observed loss of water. The ion at m/z 222 (M-60) seemed unusual for a lactone system (14). Presumably, rearrangements resulted in a loss equivalent to an acetic acid molecule, thus forming the ion $C_{16}H_{30}^{+\cdot}$

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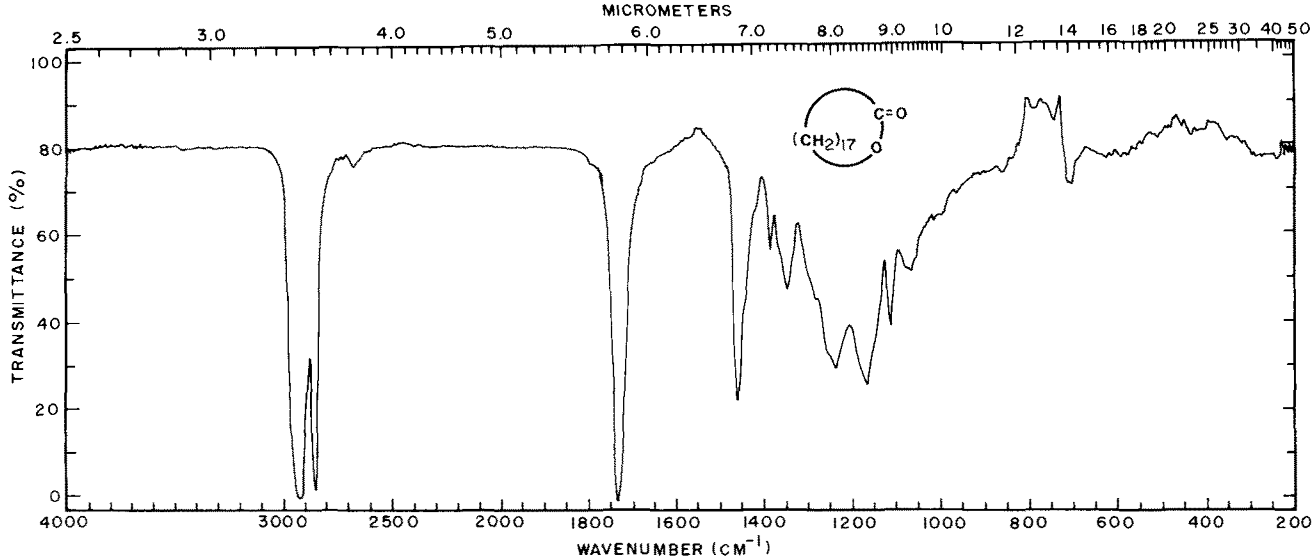

FIG. 1. IR spectrum of 18-octadecanolide.

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