The House Fly Attractants in Mushrooms

Part II. Identification of the Fraction D₃ and the Activities of Some Related Compounds

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A house fly attracting substance, referred to as D₃ in the preceeding paper,¹⁾ was identified with 1,3-diolein.

Among the related compounds, 1- and 2-monoolein and α,ω -glycol monooleate with the formula: $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_n\text{COO}(\text{CH}_2)_n\text{OH}$ ($n \leq 6$), were found to have activities ten times that of 1,3-diolein.

From the squeezed juice of the fruiting bodies of a mushroom, *Amanita muscaria* (L.) Fr., a fraction referred to as D₃ was obtained as homogeneous, colorless crystals and characterized as follows:¹⁾ mp 22~23°C.; an apparent molecular weight determined with Rast's method, 693; *Anal.* Found: C, 75.51; H, 11.54; O, 12.95 (by difference), *Calcd.* for a formula, C₃₉H₇₂O₅: C, 75.43; H, 11.69; O, 12.88%; M. W. 621; $[\alpha]_D = \pm 0$ (1.79% in chloroform); IR and NMR spectra suggesting the existence of an esterified residue of a long, straight chain, unsaturated fatty acid and a free secondary hydroxyl group, as assigned in Table I.

An intensive search for any hydroxyl esters of unsaturated fatty acids similar to D₃ in literatures revealed that the above mentioned properties of D₃ well coincide with those of 1,3-diolein. Hence 1,3-diolein was prepared according to the Daubert's established method²⁾ comprising acylating 1-monotrityl glycerol with oleoyl chloride and effecting acyl migra-

tion with dry HCl. Trityl alcohol, a by-product of the above reaction tenaciously contaminating in the end product and having complicated the identification work with the natural product at first, was found to be easily and clearly removed by a silicic acid chromatography, including washing with benzene to remove trityl alcohol and thereafter developing with 2% v/v ethyl acetate-benzene to elute 1,3-diolein selectively.

The 1,3-diolein prepared and purified as above and assured to be homogeneous by thin-layer chromatography possessed the following properties: mp 21~22°C.; *Anal.* Found: C, 74.94; H, 11.67. *Calcd.* for C₃₉H₇₂O₅, C, 75.43; H, 11.69%; IR and NMR spectra shown in Figs. 1 and 2 were found to be quite identical with those of D₃ which were shown along with.

The identity of the fraction D₃ with 1,3-diolein was further supported by the products of ozonolysis and hydrogenation.

D₃ absorbed 2.18 mols of hydrogen when reduced with 5% Pd-carbon in ethanol. The reduction product was identified with 1,3-distearin, prepared from trityl glycerol and stearoyl chloride on the basis of mixed melting

1) T. Muto and R. Sugawara, *Agr. Biol. Chem.*, **29**, 949 (1965).

2) B. F. Daubert and E. S. Lutton, *J. A. C. S.*, **69**, 1449 (1947).

TABLE I. A QUALITATIVE ASSIGNMENT OF THE NMR AND IR BANDS CHARACTERIZING THE FRACTION D₃

NMR bands* (ppm)	IR bands (cm ⁻¹)
(1) 0.90	(a) 721
(2) 1.28	(b) 1655
(3) 1.94	(c) 1743
(4) 5.28	(d) 1167
(5) 2.30	(e) 3480
(6) (7) 4.04 or 4.08	(f) 1091
(8) 4.04	

* Assigned in reference to the spectra catalog of VARIAN associates.

FIG. 1. IR Spectra of the Synthetic 1,3-Diolein (A) and Crystalline D₃ (B) in Liquid Film.

FIG. 2. NMR Spectra of the Synthetic 1,3-Diolein (A) and Crystalline D₃ (C) in CCl₄.

point and infrared spectra.

The ozonide obtained by passing ozone through an ice-cooled solution of D_3 in carbon

tetrachloride was treated with aqueous ferrous sulfate and steam-distilled into a 2,4-dinitrophenylhydrazine-HCl solution. The hydrazone was identified with that from nonyl aldehyde as had been expected.

Since the identity of D_3 with 1,3-diolein was established from the above results and the latter exhibited the same level of house fly attractancy with the former, some related compounds were prepared and their activities compared: 1-monoolein and 2-monoolein were no less than ten times as attractive as 1,3-diolein; 3-chloro- and 3-methoxy-1,2-propanediol 1-monooleate and 1,2-propanediol 1-monooleate exhibited nearly the same activities with 1,3-diolein; and, glycerol, oleic acid, methyl oleate, triolein, ethylene glycol dioleate and 1,3-distearin had no significant attractancy.

From these results it was primarily considered that at least one esterified residue of an unsaturated, long chain fatty acid together with at least one hydroxyl group, preferably primary one, should be necessary for compounds to exhibit attractancy. On this assumption, as compounds satisfying these

conditions in one of the simplest combinations, α, ω -glycol monooleates with a general formula, $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{COO}(\text{CH}_2)_n\text{OH}$ ($n=2, 3, 4, 5, 6, 7, 8$, and 10), were prepared from corresponding glycols and oleoyl chloride.

The activities of the esters with n from 2 through 6 were found at about the same level with 1-monoolein, that is, ten times higher than 1,3-diolein and a significant drop in activities was noted for the esters with $n=8$ and 10. In conclusion, a group of insect attractants represented by the formula: $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{COO}(\text{CH}_2)_n\text{OH}$ ($n \leq 6$) has been derived by modifying 1,3-diolein, an identified house fly attractant in *Amanita muscaria* and proposed as a new type of insect attractant. Results of the modification of the acid portion will be dealt with in another paper.

EXPERIMENTAL

Preparation of 1,3-diolein. A crude product prepared by the method of Daubert *et al.*²⁾ was purified by a chromatographic procedure: About 2 g of the crude product was dissolved in a small amount of benzene and placed on a column of 100 g silicic acid and trityl alcohol was washed out with about 1000 ml of benzene. Thereafter 1,3-diolein was eluted with 2% v/v ethyl acetate-benzene: The fractions containing 1,3-diolein appeared after the passage of 300 ml of the solvent and nearly completely eluted after the passage of 1000 ml of the solvent. The solvent was evaporated *in vacuo* and the residue recrystallized twice from methanol. mp 21~22°C. *Anal.* Found: C, 74.94; H, 11.67. Calcd. for $\text{C}_{36}\text{H}_{72}\text{O}_5$: C, 75.43; H, 11.69%.

Hydrogenation of the D_3 . A solution of 725 mg of D_3 in 20 ml of ethanol was hydrogenated under normal pressure and at room temperature with 1 g of 5% Pd-Carbon. After one hour absorption of hydrogen was complete (2.18 mols). The catalyst was removed by filtration, the solvent evaporated and the residue recrystallized from methanol. mp 71~73°C.

Anal. Found: C, 74.68; H, 12.05. Calcd. for $\text{C}_{39}\text{H}_{76}\text{O}_5$: C, 74.94; H, 12.26%.

The product was identified with 1,3-distearin (mp 76~77°C) prepared from stearoyl chloride and 1-monotrityl glycerol according to the Daubert's

method, on the basis of mixed melting point (71~74°C) and infrared spectra.

Ozonolysis of D_3 . Ozone was passed through a solution containing 144 mg of D_3 in 10 ml of carbon tetrachloride for about 6 hr. The solvent was removed with slight warming and the residue treated with 50 ml of water containing 1 g of FeSO_4 . The precipitate was removed and the filtrate was steam-distilled into a saturated solution of 2,4-dinitrophenylhydrazine in 3.6% aqueous HCl. The precipitate after having been left to stand overnight was collected by filtration, washed with water and dried *in vacuo*. The crude product was purified through a silicic acid chromatography using benzene as an eluant and finally recrystallized from ethanol. mp 98~99°C.

Anal. Found: N, 16.87. Calcd. for $\text{C}_{15}\text{H}_{22}\text{N}_4\text{O}_4$: N, 17.38%. λ_{max} 359 m μ (a, 77.4).

The product was identical with the 2,4-dinitrophenylhydrazone of nonyl aldehyde (bp 82°C/4 mmHg) which had been prepared by treating nonyl bromide with trimethylamine N-oxide according to the method of Volker *et al.*³⁾ on the basis of mixed melting point, the mp of the synthetic sample 104°C, and the mixed melting point 101°C, and λ_{max} of synthetic: 359 m μ (a, 79.6), and infrared spectra.

Preparation of the compounds related to 1,3-diolein. 1-Monoolein was prepared from acetone-glycerol according to the method of Daubert *et al.*,⁴⁾ mp 30~33°C.; 2-monoolein from benzylidene glycerol and oleoyl chloride followed by decomposition with boric acid in triethyl borate according to the method of Martin,⁵⁾ mp 28~30°C; 1,3-distearin from 1-trityl glycerol and stearoyl chloride, mp 76~77°C; 1,2-propanediol 1-monooleate from the diol and oleoyl chloride, bp 196~198°C (2 mmHg); glycerol 3-monochlorohydrin 1-monooleate from glycerol monochlorohydrin and oleoyl chloride in quinoline, bp 273~275°C (1 mmHg); and 3-methoxy glycerol 1-monooleate by treating 3-monochlorohydrin 1-monooleate with sodium methoxide in methanol, bp 202~206°C (1 mmHg).

Preparation of ethylene glycol monooleate. To a solution containing 9 g of oleoyl chloride and 3 g of ethylene glycol (about 0.8 molar excess) in 22 ml

3) F. Volker and O. Sören, *Chem. Ber.*, **94**, 1360 (1961).

4) B. F. Daubert, H. H. Fricke and H. E. Longenecker, *J. A. C. S.*, **65**, 2142 (1943).

5) J. B. Martin, *ibid.*, **75**, 5482 (1953).

of dry chloroform was gradually added 6 ml of dry quinoline. The reaction mixture was left to stand at room temperature for 1 hr, thereafter refluxed for 4 hr. The reaction mixture was cooled, diluted with 50 ml of chloroform and successively washed with 2 N H_2SO_4 , cold water, 5% aqueous Na_2CO_3 and cold water. The solvent was removed, dissolved in a small amount of benzene and placed on a column of 100 g alumina. After washing with benzene, the monooleate was eluted with about 1000 ml of 5% v/v methanol-benzene. The solvent was evaporated and the residue distilled. bp $198\sim 200^\circ\text{C}$ (2 mmHg). Yield: 3.7 g (34%). *Anal.* Found: C, 73.54; H, 11.63. Calcd. for $\text{C}_{20}\text{H}_{38}\text{O}_3$: C, 73.57; H, 11.73%.

Preparation of α,ω -glycol monooleate. According to the just described method, the following monoesters were prepared from the corresponding α,ω -glycols: propylene glycol monooleate, bp $206\sim 207^\circ\text{C}$ (2 mmHg), yield 23.5%; tetramethylene glycol monooleate, bp $210\sim 215^\circ\text{C}$ (2 mmHg), yield 16.3%; pentamethylene glycol monooleate, bp $218\sim 222^\circ\text{C}$ (2 mmHg), yield 28.5%; hexamethylene glycol monooleate, bp 230°C (2 mmHg), yield 15.7%; octamethylene glycol monooleate, bp $284\sim 285^\circ\text{C}$ (3 mmHg), yield 14.8%; decamethylene glycol monooleate, mp 23°C , yield 25.7%.

House fly attracting activities of 1,3-diolein and related compounds; Typical examples of the house fly attracting tests repeatedly carried out with the above prepared compounds according to the previously reported procedure¹⁾ will be described below: To petri-dishes, 9 cm in diameter, containing 50 ml water were respectively added 10 mg and 1 mg of the fraction D_3 and 1,3-diolein and allowed to stand in the test room with about 1000 flies from a batch having started to hatch before 5 days. The captured flies were 27, 11, 30 and 9 after 16 hr. In another similar experiment carried out with 50 mg of glycerol, 10 mg each of triolein, 1,3-diolein, glycerol 3-monochlorohydrin 1-monooleate, 1,3-distearin, 1,2-propanediol 1-monooleate and 3-methoxy glycerol 1-monooleate and 1 mg each of 1-monoolein and 2-monoolein, the drowned flies were 1, 1, 16, 15, 0, 17, 16, 33, and 19 respectively in the mentioned order. In still another experiment using 1 mg each of 1-monoolein, 1,3-diolein, ethylene glycol monooleate, ethylene glycol dioleate, methyl oleate and

100 mcg each of ethylene glycol monooleate and 1-monoolein, the captured flies were 24, 6, 22, 1, 4, 8 and 12 respectively.

House-fly attracting activities of α,ω -glycol monooleates. To petri-dishes, 9 cm in diameter, containing 50 ml of water were added 1 mg each of glycol 1-monooleate, 1,3-trimethylene glycol monooleate, 1,4-tetramethylene glycol monooleate, 1,5-pentamethylene glycol monooleate, 1,6-hexamethylene glycol monooleate, 1,8-octamethylene glycol monooleate and 1,10-decamethylene glycol monooleate and tested as above. The drowned flies after 16 hr were 31, 33, 20, 28, 29, 5 and 2 in the mentioned order.

House-fly attracting activities of ethylene glycol monooleate. (a) At one end of the shelf in the test room, milk and wicked water supply were placed. At a distance of 1.5 m was placed a petridish, 15 cm in diameter, filled with 300 ml of water and further 1.5 m apart was placed 500 mg of the ester dropped to 300 ml of water.

Of about 700 flies having been released in the room, 311 and 31 respectively fell onto the basins of the ester and water, while about 150 flies still remained swarming over the diet, after 3 days.

(b) Two cylindrical plastic traps, 22 cm in height and 12 cm in diameter, provided with a caving-in, inverted funnel-like bottom provided with an opening, 3 cm in diameter, in the centre were employed. Petri-dishes, 9 cm in diameter, were attached to the bottom at a distance of about 2 cm downward with cellophane tape. About 10 ml of the ester was spread in the petri-dish attached to one trap, while the petri-dish attached to another being left empty for comparison. Furthermore, about 100 ml of water containing 1 g of detergent powder were poured in each of the traps to capture the flies having entered into the traps from the bottom. The containers thus prepared were hanged on the wall of the test room with about 500 flies released, separated about 2 m and the places were mutually changed every day. After one week, 106 and 26 flies were captured respectively in the test and control traps.

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