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Unique occurrence of fenchol in the animal kingdom[1]

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Summary. (−)-β-Fenchol, a compound occurring in plants but not heretofore reported to occur in an animal product, was isolated from volatiles released by adult male Mediterranean fruit flies, *Ceratitis capitata* Wiedemann, with the male's previously identified sex pheromones. It neither attracts females nor synergizes the pheromones, and its function remains unknown.

The Mediterranean fruit fly, *Ceratitis capitata* Wiedemann, or 'medfly', is one of the most destructive pests of citrus fruits in Hawaii, Central America and the Mediterranean countries of Europe and the Middle East. 6 years ago[3] we isolated 2 sex pheromones, (*E*)-6-nonen-1-ol and methyl (*E*)-6-nonenoate (MEN), from a cold-trap condensate of the volatiles of laboratory-reared male medflies following collection into methylene chloride. These compounds were separately attractive and sexually excitatory to virgin females in small laboratory cages, but in large outdoor cages a combination of both pheromones in equal amounts plus certain fatty acids, also produced by the males, was required to attract females[3,4]. Moreover, when 2 of the most promising combinations were tested in the field, they attracted large numbers of males, but no females[4]. Subsequently we found that MEN was, in fact, solely responsible for the outstanding attraction of the combinations to male medflies in the field[5,6]. We theorized that we might have overlooked or failed to obtain by our previous method of collecting volatiles, some chemical substance that might be required to trigger female response in the field or to synergize the pheromones we had already isolated. We report here the isolation, from male medfly volatiles, of fenchol released simultaneously with the sex pheromones.

A stream of air (2–3 l/min) was passed for 126 h through a wire-screen cage containing 4000 3–15-day-old calling live male laboratory-reared medflies and enclosed in a plexiglas box. The volatiles-laden air was then passed through a tube packed with 50/80 mesh Porapak Q, the adsorbent was extracted with ethyl ether, and the extract was separated into acidic and neutral portions. Gas chromatograms of the methyl esters prepared from the acids were identical with those obtained previously[4]. Silica gel chromatography (Bio-Sil HA, minus 325 mesh) of the neutral portion (89.1 mg) and gas chromatography-mass spectrometry (GC-MS) (Hewlett-Packard 5992A, 2% OV 101 on 100/120 mesh Chromosorb WHP, 100 °C) of the eluted components gave 62.3 mg of MEN[3] eluted with hexane-ether (95:5) and a mixture consisting of 20.1 mg of (*E*)-6-nonen-1-ol[3] and 6.7 mg of colorless liquid with a camphoraceous odor, eluted with hexane-ether (75:25). This mixture was readily separable by preparative thick-layer chromatography on DC 550 silicone oil-impregnated silica gel G. eluting the plates with methanol-H_2O (70:30)[7]; R_f-values were 0.55 and 0.40, respectively.

The colorless liquid showed an IR-spectrum indicative of a cyclic terpene alcohol. The mass spectrum showed a base peak (m/e) of 81.2 and a molecular ion at 154.1; on comparison with standard reference spectra[8] it appeared to be identical with that of fenchol[9]. The NMR-spectrum was identical with that reported for fenchol by Musher[10]. Fenchol ($C_{10}H_8O$) may exist in the α-form *(endo)* or the β-form *(exo)*[11,12]. The isolated compound was levorotatory, $[\alpha]_D^{25}$ −23.33° (5% in ethanol), which compared very well with literature values[13,14] (−23.28°, −23.38°) reported for (−)-β-fenchol (1*S*-*exo*-1,3,3-trimethylbicyclo-[2.2.1]heptan-2-ol).

The identity of the compound as (−)-β-fenchol was confirmed by synthesizing the authentic material according to a modification of the method of Coulombeau and Rassat[15], using a reflux period of 6 rather than 20 days. The resultant mixture of α- and β-fenchols was converted to the acid phthalates, which were recrystallized from hexane and saponified to give the free alcohols. The latter were converted to the 3,5-dinitrobenzoate and then resaponified to give pure (−)-β-fenchol boiling at 91 °C at 18 mm, m.p. 6 °C, n_D^{25} 1.4778, $[\alpha]_D^{25}$ −23.33° (5% in ethanol); it was identical (IR, GC-MS, NMR, optical rotation) with the compound obtained from male medfly volatiles.

Fenchol has been reported as a constituent in the essential oils of a number of plants of various families: leaves of *Artemisia santolinaefolia* Turcz. ex Bess. (family Compositae)[16]; wood of *Chamaecyparis lawsoniana* (A. Murr.) Parl. (family Cupressaceae)[17]; leaves of *Baekea frutescens* L. (family Myrtaceae)[18]; *Dalbergia parviflora* Roxb. (family Leguminosae)[18]; leaves of *Agathis australis* (Lamb.) Steud., twigs of *Picea glauca* (Moench) Voss., and roots of *Pinus* spp. (family Pinaceae)[19–21]; seeds of *Clausena anisata*

(Willd.) Hook. f., and berries of *Luvunga scandens* (Roxb.) Buch.-Ham. (family Rutaceae)[22,23]; and roots of *Foeniculum vulgare* Mill., *Selinum vaginatum* C.B. Clarke, and *Seseli sibiricum* Benth. (family Umbelliferae)[18,24,25]. Only in *L. scandens* and *C. lawsoniana* has the fenchol been reported to be optically active; the l-β-isomer has been definitely identified in *L. scandens*.

Fenchol has never before been reported in an animal product. Its occurrence in completely laboratory-reared male medflies was totally unexpected, since their rearing diet[26] contains no plant material other than wheat shorts. A steam distillate of wheat shorts showed no trace of fenchol by spectral analyses and consisted almost completely (92%) of 2-acetylpyrazine[3]. (−)-β-Fenchol was unattractive to either sex of medflies in laboratory tests and in the field on the infested islands of Maui and Hawaii. Combinations with MEN + (*E*)-6-nonen-1-ol + medfly acids in their natural proportions also failed to attract females in the field.

1 Mention of a proprietary product in this paper does not constitute a recommendation or an endorsement of the product by the US Department of Agriculture.

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3 M. Jacobson, K. Ohinata, D.L. Chambers, W.A. Jones and M.S. Fujimoto, J. med. Chem. *16*, 248 (1973).
4 K. Ohinata, M. Jacobson, S. Nakagawa, M. Fujimoto and H. Higa, J. envir. Sci. Health *A 12*, 67 (1977).
5 K. Ohinata, M. Jacobson, S. Nakagawa, T. Urago, M. Fujimoto and H. Higa, J. econ. Ent. *72*, 648 (1979).
6 K. Ohinata, M. Jacobson and S. Nakagawa, U.S. Patent 4,152,422, May 1, 1979.
7 E. Stahl, ed., Thin-Layer Chromatography, 2nd edn, p.226. Springer Verlag, New York 1969.
8 E. Stenhagen, S. Abrahamsson and F.W. McLafferty, in: Registry of Mass Spectral Data. Wiley and Sons, New York 1974.
9 B. Willhalm, A.F. Thomas and M. Stoll, Acta chem. scand. *18*, 1573 (1964).
10 J.I. Musher, Molec. Phys. *6*, 93 (1963).
11 T.K. Devon and A.I. Scott, in: Handbook of Naturally Occurring Compounds, vol.II, Terpenes, p.32. Academic Press, New York 1972.
12 W. Klyne and J. Buckingham, in: Absolute Configurations of Organic Molecules, vol.1, 2nd edn. Oxford University Press, New York 1978.
13 J. Kenyon and H.E.M. Priston, J. chem. Soc. *127*, 1472 (1925).
14 W. Hückel, H. Kindler and H. Wolowski, Ber. dt. chem. Ges. *77*, 220 (1944).
15 A. Coulombeau and A. Rassat, Bull. Soc. Chim. Fr. *1965*, 3338.
16 M.I. Goryaev, G.K. Kruglykhina, M.G. Pugachev and I.M. Shabanov, Izv. Akad. Nauk kazakh. SSR, Ser. Khim. *9*, 33 (1956).
17 G. Kritchevsky and A.B. Andersson, J. Am. pharm. Assoc. *44*, 535 (1955).
18 E. Guenther and D. Althausen, in: The Constituents of Essential Oils, vol.II, p.252. Van Nostrand, New York 1949.
19 J.R. Hosking. Rec. trav. chim. Pays-Bas *48*, 622 (1929).
20 G. Komppa, Ann. Acad. Sci. Fenn. A *38*, 8 p (1933).
21 L.M. Humphry, Trans. Inst. chem. Engrs *9*, 40 (1931).
22 S. Talalaj, W. Afr. Pharm. *7*, 44 (1965).
23 K.K. Baslas, Perfum. essent. Oil Rec. *50*, 124 (1959).
24 K.L. Handa, M.C. Nigam and M.L. Sharma, Riechstoffe Aromen *13*, 181 (1963).
25 K.L. Handa, D.L. Smith and L. Levi, Perfum. essent. Oil Rec. *53*, 607 (1962).
26 N. Tanaka, L.F. Steiner, K. Ohinata and R. Okamoto, J. econ. Ent. *62*, 967 (1969).

Metabolism in Porifera XII. Further informations on the biosynthesis of 3β-hydroxymethyl-A-nor-steranes in the sponge *Axinella verrucosa*

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Summary. Direct incorporation and cold trap experiments suggest that cholest-4-en-3-one is an intermediate in the conversion of cholesterol into 3β-hydroxymethyl-A-nor-cholestane in the marine sponge *Axinella verrucosa*. Cholest-4-en-3-one is further transformed by the sponge into cholest-4-en-3β-ol, 5α-cholestan-3-one and 5α-cholestan-3β-ol; these compounds arise from side reactions, which are not part of the major metabolic route leading to 3β-hydroxymethyl-A-nor-steranes.

We have previously shown that the conversion[1] of cholesterol (**1**) into 3β-hydroxymethyl-A-nor-cholestane (**2**) by the sponge *Axinella verrucosa*, which contains as the sole sterol components a novel group of A-nor-stanols[2], involves the formation of a C-C linkage between the C-4 and C-2 of cholesterol, while the C-3 provides the hydroxymethyl carbon[3], and there is a loss of the 3α- and 4β-hydrogen atoms of cholesterol[4]. On these results a 3-oxo-Δ^4-steroid was also postulated[4] as an intermediate.

This paper presents the results of an investigation undertaken to examine the intermediacy of cholest-4-en-3-one (**3**) and other supposed intermediates in the above-mentioned conversion.

Materials and methods. [4-^{14}C]-Cholesterol (57 mCi/mmole) was supplied by the Radiochemical Centre, Amersham (Bucks., Great Britain). [4-^{14}C]-Cholest-4-en-3-one was prepared by an Oppenauer oxidation[5] of [4-^{14}C]-cholesterol and purified by preparative TLC.

Feeding experiments (10-day incubations) and the extraction of the metabolites were performed as previously described[1,3]. The weights of the extracts of each experiment are reported in the tables; from each g of the crude extracts 200–250 mg of 3β-hydroxymethyl-A-nor-steranes were recovered.

The extracts were chromatographed on a silica gel column (benzene-diethyl ether), after addition of the appropriate carrier material, and the metabolites were purified to a constant specific activity as follows. Cholesta-4-en-3-one (**3**), 5α-cholestan-3-one (**4**) and cholesterol (**1**) were purified by preparative TLC and crystallization. To 5α-cholestan-3β-ol (**5**) fractions 3-chloroperbenzoic acid was added to remove any unsaturated contaminant[6] and then 5α-cholestan-3β-ol (**5**) was recovered by preparative TLC (benzene-diethyl ether, 1:1) and purified by crystallization. 3β-Formyl-A-nor-steranes (partial structure **6**) were purified by preparative TLC and oxidation to the correspond-