Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXXVIII, No. 10

SYNTHESIS OF TRITIATED SEX PHEROMONES OF THE PROCESSIONARY MOTH *THAUMETOPOEA PITYOCAMPA* AND THE EGYPTIAN ARMYWORM *SPODOPTERA LITTORALIS*

Angel Guerrero* and Joan Feixas

Department of Biological Organic Chemistry, C.I.D. (CDIC)

Jordi Girona, 18-26. 08034-Barcelona, Spain

Summary. Synthesis of tritiated sex pheromones of the processionary moth *Thaumetopoea pityocampa* and the Egyptian armyworm *Spodoptera littoralis* has been accomplished by a simple route involving tritiated sodium borohydride reduction of the corresponding aldehyde followed by acetylation of the resulting radiolabelled alcohol. The process occurs with high chemical and radiochemical yields and the compounds have been used in pheromone catabolism studies.

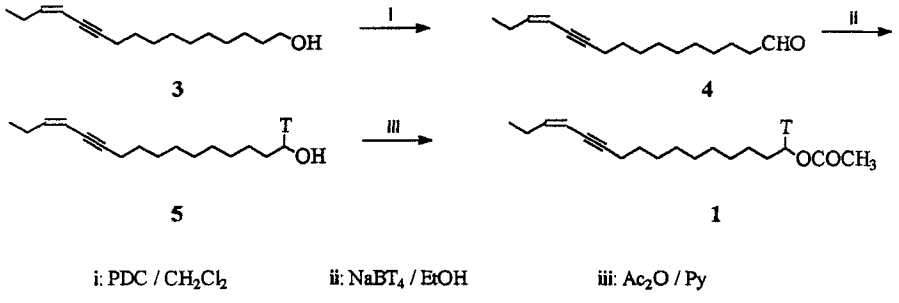
Key words: tritiated pheromone, synthesis, processionary moth, *Thaumetopoea pityocampa*, Egyptian armyworm, *Spodoptera littoralis*.

Pheromone binding and catabolism is an area of research which has gained increasing interest in the last few years (1). Studies in this subject require the presence of isotopically-labelled molecules with isotopes of very similar spatial and electronic features to hydrogen, like tritium, to avoid undesired steric or stereoelectronic interactions with the binding or catabolic enzymes. In general, radioactive labels have been introduced at distant locations from the metabolic site, for instance in the preparation of tritiated pheromones of *Antheraea*

CCC 0362–4803/96/010929–05

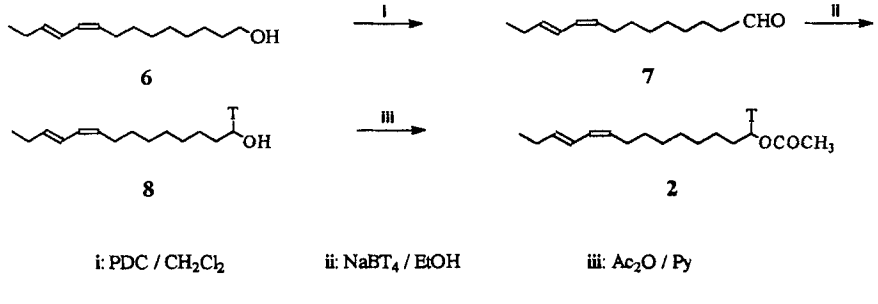
Received 15 April 1996
Revised 22 April 1996

polyphemus (2), *Plutella xylostella* (3), *Bombyx mori* (4) or *Heliothis virescens* (5), among others. However, to our knowledge, relatively few examples of tritiated pheromones with the radiolabel at nearby positions to the metabolic site of the pheromone have been described (6-8), because of the possibility of concomitant loss of radioactive label during the pheromone degradation process (1).



Scheme 1

In this paper we report on the preparation for the first time of the tritiated pheromones of the processionary moth *Thaumetopoea pityocampa* (1-^{3}H)-(Z)-13-hexadecen-11-ynyl acetate (**1**), and the Egyptian armyworm *Spodoptera littoralis* (1-^{3}H)-(Z,E)-9,11-tetradecadienyl acetate (**2**), in a straightforward manner and good overall chemical and radiochemical yields, from the corresponding unlabelled alcohols. The pheromones have been radiolabelled at the carbon bearing the acetate group by sequential oxidation to the adehydes **4** and **7**, followed by tritiated sodium borohydride reduction to furnish labelled alcohols **5** and **8**, and acetylation (Schemes 1 and 2). The synthetic radiolabelled pheromones have been used in studies of inhibition of antennal esterases of the insects (9, 10) with no appreciable loss of radioactivity in the catabolism process of the pheromones.



Scheme 2

MATERIALS AND METHODS

IR spectra were recorded on a Bomem MB-120 with Fourier transform spectrophotometer. ^{1}H and ^{13}C NMR spectra were obtained in $CDCl_3$ solutions on a Varian Gemini 200 spectrometer, operating at 200 MHz for ^{1}H and 50 MHz for ^{13}C. The values are expressed in δ scale relative to tetramethylsilane. GLC analyses were performed on a Carlo Erba 4130 gas chromatograph, equipped with a FID detector and using a SE-54 50 m x 0.32 μm ID fused silica capillary column. Thin layer chromatography was performed on silica gel 60 F_{254} plates (Merck). Unlabelled materials were visualized with iodine and by UV irradiation (254 nm). Radioactive spots were visualized with a RITA TLC Radioscanner (Isomess IM 3000, Germany). Vials containing radioactive material were diluted with 4 ml of Unisolve scintillation liquid and counted on a Kontron Betamatic scintillation counter. Column chromatography purifications were performed on silica gel 60-200 μm (Merck) in disposable Pasteur pipettes. Chemicals were obtained from comercial sources. Anhydrous CH_2Cl_2 was prepared by distillation from P_2O_5 and anh. pyridine was obtained by distillation from CaH_2.

(Z)-13-hexadecen-11-ynal (4). To a solution of 100 mg (0.42 mmol) of alcohol **3**, prepared by stereospecific coupling of (*Z*)-1-bromo-1-butene with 2-(11-dodecynyloxy)tetrahydropyran (11), in 2 ml of anh. CH_2Cl_2 was added 233 mg (0.63 mmol) of pyridinium dichromate (0.63 mmol) at room temperature. The reaction mixture was stirred for 24 h, filtered through Celite and washed with CH_2Cl_2. The solvent was evaporated off and the residue purified by column chromatography on silica gel eluting with hexane:ethyl acetate mixtures to furnish aldehyde **4** in 60% yield. IR ν 3021, 1727, 1611, 954, 720 cm^{-1}. ^{1}H NMR δ 9.74 (t J=1.9 Hz, 1H, CHO), 5.78 (dt J=10.6Hz, J'=7.2 Hz, 1H, C<u>H</u>=CHC≡C), 5.38 (dm J=10.6 Hz, 1H, CH=C<u>H</u>C≡C), 2.40 (dt J=7.2 Hz, J'=1.9 Hz, 2H, C<u>H</u>$_2$CHO), 2.29 (c, 4H, CH_2C=C and C≡CCH_2), 1.7-1.1 (b, 14H, 7CH_2), 0.98 (t J=7.6 Hz, 3H, CH_3). ^{13}C NMR δ 202.8 (C-1), 143.9 (C-14), 108.6 (C-13), 94.3 (C-11), 77.1 (C-12), 43.8 (C-2), 29.2-28.7, 25.9, 23.3, 22.0, 19.4, 13.4 (C-16).

(1-^{3}H)-(Z)-13-Hexadecen-11-ynol (5). This compound was prepared by reduction of aldehyde **4** (8 mg) with tritiated sodium borohydride (specific activity 468 mCi/mmol) (25 mCi) in ethanol in 58.4% radiochemical yield (12).

(1-^{3}H)-(Z)-13-Hexadecen-11-ynyl acetate (1). Tritiated alcohol **5** was treated with 300 µl of anh. pyridine and 20 µl of acetic anhydride. The reaction mixture was stirred at room temperature for 5 h and concentrated under nitrogen. The residue was taken up in hexane and purified by column chromatography on silica gel eluting with hexane:ether mixtures. Fractions containing tritiated acetate **1** were combined, concentrated under nitrogen and diluted with ethanol to obtain a $9.6x10^{-2}$M stock solution with a specific activity of 120 mCi/mmol.

(Z,E)-9,11-Tetradecadienal (7). Following the same procedure as described for aldehyde **4**, compound **7** was obtained in 64% yield after purification by column chromatography on silica gel. IR ν 1726, 1650, 981 cm^{-1}. ^{1}H NMR δ 9.76 (t J=1.8 Hz, 1H, CHO), 6.26 (dd J=15 Hz, J'=10.9 Hz, 1H, CH=C<u>H</u>CH=CH), 5.92 (dd J=J'=10.9 Hz, 1H, CH=CHC<u>H</u>=CH), 5.67 (dt J=15 Hz, J'=6.6 Hz, 1H, C<u>H</u>=CHCH=CH), 5.26 (dt J=10.8 Hz, J'=7.5 Hz, 1H, CH=CHCH=C<u>H</u>), 2.42 (dt J=7.3 Hz, J'=1.9 Hz, 2H, C<u>H</u>$_2$CHO), 2.14 (m, 4H, C<u>H</u>$_2$CH=CHCH=CHC<u>H</u>$_2$), 1.62 (m, 2H, C<u>H</u>$_2$CH$_2$CHO), 1.36 (m., 2H, C<u>H</u>$_2$CH$_2$CH$_2$CHO), 1.29 (b, 6H, 3CH$_2$), 1.01 (t J=7.5 Hz, 3H, CH$_3$). ^{13}C NMR δ 203.0 (C-1), 136.2 (C-12), 130.1 (C-9), 128.6 (C-10), 124.6 (C-11), 43.9 (C-2), 29.6-29.1, 27.6, 25.9, 22.0, 13.7 (C-14).

(1-^{3}H)-(Z,E)-9,11-Tetradecadienol (8). A mixture of 11.3 mg (0.054 mmol) of aldehyde **7**, 1.5 ml of ethanol and 0.1 ml of a 0.01N NaOH soln. in ethanol was added via syringe to tritiated sodium borohydride (468 mCi/mmol). After 3 h of stirring at room temperature no starting aldehyde was detected by TLC. The ethanol was removed under a gentle stream of nitrogen and the residue purified by column chromatography eluting with hexane-ether mixtures. Fractions containing radioactive alcohol **8** were combined and concentrated to prepare a 10^{-2} M soln. in hexane.

(1-^{3}H)-(Z,E)-9,11-Tetradecadienyl acetate (2). The solution containing tritiated alcohol **8** was freed from solvent and following the same procedure as described for compound **1** the desired tritiated acetate **2** was obtained (7.1 mg, 51.8 % overall yield from aldehyde **7**), after

purification by column chromatography eluting with hexane-ether mixtures. The specific activity was 137 mCi/mmol (radiochemical yield 77.6%). The tritiated acetate was dissolved in ethanol to obtain a 5.64×10^{-2} M stock solution for biological assays.

ACKNOWLEDGEMENTS

We gratefully acknowledge CICYT (PB 93-0158 and AGF 95-0185) for financial support and CIRIT (Generalitat de Catalunya) for a predoctoral fellowship to J.F. Part of this work was conducted at Stony Brook and we are indebted to Prof. G.D. Prestwich for laboratory space and facilities. We also thank I. Durán for assistance.

REFERENCES

1. Prestwich G.D. - Pheromone Biochemistry (G.D. Prestwich and G.J. Blomquist, eds), Academic Press, N.Y., pp. 473-527 (1987).
2. Vogt R.G., Riddiford L.M. and Prestwich G.D. - Proc. Natl. Acad. Sci. USA **82**:8827 (1985).
3. Prestwich G.D. and Streinz L. - J. Chem. Ecol. **14**:1003 (1988).
4. Kasang, G. - Z. Naturforsch B **23**:1331 (1968).
5. Ding Y.-S. and Prestwich G.D. - J. Chem. Ecol. **12**:411 (1986).
6. Sheads R.E. and Beroza M. - J. Agric. Food Chem. **21**:751 (1973).
7. Ferkovich S.M. - Perception of Behavioral Chemicals (D.M. Norris ed), Elsevier/North Holland, Amsterdam, pp. 167-185 (1982).
8. Prestwich G.D., Graham S.McG., Kuo J.-W. and Vogt R.G. - J. Am. Chem. Soc. **111**:636 (1989).
9. Durán I., Parrilla A., Feixas J. and Guerrero A. - Bioorg. Med. Chem. Lett. **3**:2593 (1993).
10. Durán I. and Guerrero A., unpublished.
11. Michelot D., Guerrero A. and Ratovelomanana V. - J. Chem. Res. (S) 93 (1982).
12. Feixas J., Prestwich G.D. and Guerrero A. - Eur. J. Biochem. **234**:521 (1995).