the presence of a Y chromosome suggests that these cells were derived from a male. Third, the ultrastructure of MA 160 differs from that of HeLa cells. The well-developed rough endoplasmic reticulum and the presence of desmosomes are findings that generally have not been found in HeLa cells (*10*). Fourth, Robertson (*11*) has found differences in the alkaline phosphatase content between MA 160 and HeLa cells. Fifth, the fact that these cells form glandlike structure in the cheek pouch and subcutaneous tissues of immune-suppressed hamsters supports their being derived from prostatic epithelium. HeLa cells also produce solid tumors under similar conditions but pseudo-gland formation has not been reported. Attempts by us to demonstrate pseudo-gland formation in tumors produced by the injection of suspensions of HeLa cells into the cheek pouch of immune-suppressed hamsters were unsuccessful.

Almost nothing is known about the etiology and natural history of carcinoma of the prostate in man. For example, there is considerable debate as to whether cancer of the prostate arises de nova from normal prostatic cells or whether these tumors develop gradually with benign hyperplastic epithelium as an intermediate stage. Previously it was not possible to study the biology of single human prostatic cells since this tissue had not been grown as a monolayer culture. Recently, however, not only MA 160 but also other benign and malignant human prostatic tissue has been propagated as cell cultures and cell suspensions (*7*). If, when these techniques are used, epithelium from benign prostatic adenomas consistently undergoes spontaneous in vitro neoplastic transformation it would suggest that these cells are premalignant. Further work is needed to resolve this question as a basis for further research in this area.

ELWIN E. FRALEY*
SIDNEY ECKER
National Cancer Institute, National Institutes of Health, Bethesda, Maryland, and *Department of Surgery, University of Minnesota, Minneapolis 55455*
MONROE M. VINCENT
Microbiological Associates, Inc., Bethesda, Maryland

References and Notes

1. L. Hayflick, *Sci. Amer.* **218**, 32 (1968).
2. K. K. Sanford, *J. Nat. Cancer Inst. Monogr.* **26**, 387 (1967).
3. H. Eagle, *Science* **130**, 432 (1959).
4. Grand Island Biological, Grand Island, N.Y.
5. Microbiological Association, Inc., Bethesda, Md.
6. D. F. Brandes and E. E. Fraley, in preparation.
7. E. E. Fraley, S. Ecker, M. M. Vincent, unpublished data.
8. S. M. Gartler, *Nature* **217**, 750 (1968).
9. R. H. Bottomley, A. T. Trainer, M. J. Griffin, *J. Cell Biol.* **41**, 806 (1969); E. H. Y. Chu and N. H. Giles, *J. Nat. Cancer Inst.* **20**, 383 (1958).
10. M. A. Epstein, *J. Biophys. Biochem. Cytol.* **10**, 153 (1961); H. W. Fisher and T. W. Cooper, *J. Cell Biol.* **34**, 569 (1967); B. A. Warren *et al., Angiologica (Basel)* **6**, 32 (1969).
11. A. L. Robertson, Jr., personal communication.

* Reprint request should be sent to E.E.F. at the University of Minnesota.

30 July 1970

Sex Pheromones of the Southern Armyworm Moth: Isolation, Identification, and Synthesis

Abstract. *Two sex pheromones have been isolated from the female southern armyworm moth,* Prodenia eridania *(Cramer), and identified as* cis-*9-tetradecen-1-ol acetate, identical with the sex pheromone of the fall armyworm moth,* Spodoptera frugiperda *(J. E. Smith), and* cis-*9,*trans-*12-tetradecadien-1-ol acetate.*

The larval stage of the southern armyworm moth, *Prodenia eridania* (Cramer), is a climbing cutworm which is an occasional pest of vegetable crops in the South (*1*). In 1962, Butt (*2*) showed that the female southern armyworm moth possesses a sex pheromone for males which is apparently produced in the tips of the abdomen; extraction of the tips with ethanol or methylene chloride removed the attractant and gave an active extract. He also reported that the adult males respond to the attractant only during night hours.

Southern armyworms reared in the laboratory at Brownsville, Texas, are sexed in the pupal stage and placed in separate cages. Virgin females are used as a source of the sex pheromone and males are used in bioassay (*3–5*). Techniques were developed to assay for the presence of the pheromone (*6*). The most satisfactory method of bioassay is to subject caged male moths, under photographic darkroom conditions, to the vapors expelled from a glass pipette treated with the test material. The males readily respond with a characteristic dance when the test material is active. The isolation procedure described herein was monitored by means of this test.

Abdomens (132,000) of virgin female moths were originally extracted with ethanol, but ethyl esters, apparently formed during extraction, made subsequent purification steps difficult. Subsequently 177,000 more abdomens were extracted with methylene chloride or hexane. The extract was dissolved in 20 volumes of acetone, and the solution was kept overnight at −10°C. The acetone, containing a large amount of inactive, precipitated white solid was filtered rapidly through a cold Büchner funnel. After being washed with cold acetone, the solid was dissolved, precipitated, filtered, and washed twice more. The combined mother liquors and washings were freed of solvent at 20 mm-Hg (bath below 40°C). At this point, the material originally extracted with ethanol, and contaminated with ethyl esters, was saponified with alcoholic KOH, and the inactive neutral material obtained therefrom was acetylated with acetyl chloride to give additional active material that was handled separately.

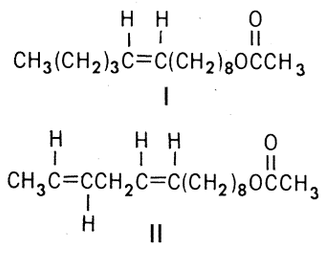
All active material was subjected to the sweep codistillation technique developed by Storherr *et al.* (*7*) for the cleanup of oils in the determination of pesticides. The procedure consisted essentially of subjecting a heated sample, coated on glass beads, to a flow of nitrogen and an intermittent flow of hexane, which removed the active material from the bulk of the sample. Active material thus obtained was chromatographed on a column of Florisil (*8*) and eluted successively with hexane and then with 3 percent ethyl ether in hexane. The latter solvent removed the active material, which was then chromatographed on a column of silica gel impregnated with silver nitrate (*9*) and eluted successively with hexane and 5, 10, and 25 percent ethyl ether in hexane. The latter two eluates, which contained all the activity, were combined and subjected to preparative gas chromatography on an OV-1 column (*10*). The active material emerged from the column as a single peak with a retention time of 5 minutes. It was subjected to preparative gas chromatography on a diethylene glycol succinate (DEGS) column (*11*), giving two active components with retention times of 9.4 minutes (compound **I**) and 12.4 minutes (compound **II**).

In approximately 20 percent of the

active material from the Florisil column, compound **I** was separated from compound **II** by fractionation of the eluate in 10 percent ether in hexane from the silver nitrate chromatography. It was then only necessary to separate compound **II** from a small amount of inactive material by preparative gas chromatography on OV-1 (*10*). Approximately 3.9 mg of compound **I** and 0.8 mg of compound **II** were obtained from 305,000 abdomens of virgin female moths.

The infrared spectrum (CCl_4 solution) of compound **I** showed strong aliphatic and carbonyl absorption and weak olefinic absorption, a C–O stretching band at 1235 cm^{-1}, characteristic of an acetate, and the absence of a band at 960 cm^{-1}, indicating that there were no *trans* double bonds present in the molecule. The mass spectrum (*12*) was typical of normal long-chain aliphatic compounds and showed a rather intense $M^+ - 60$ peak at *m/e* (mass to charge) 194 corresponding to the ion formed by the loss of acetic acid from the molecular ion of the acetate of a 14-carbon alcohol having one double bond. The nuclear magnetic resonance (NMR) spectrum (*13*) confirmed that compound **I** was a tetradecenyl acetate. Ozonolysis by the method of Beroza and Bierl (*14*) showed that the double bond was in the 9-position. The only structure consistent with these data is *cis*-9-tetradecen-1-ol acetate, identical with the sex pheromone of the fall armyworm moth [*Spodoptera frugiperda* (J. E. Smith)] (*15*).

The infrared spectrum of compound **II** showed strong aliphatic and carbonyl absorption and weak olefinic absorption; a C–O stretching band at 1220 cm^{-1}, characteristic of an acetate with greater unsaturation than that of compound **I**, and a C–H bending band at 960 cm^{-1} indicative of *trans* unsaturation. The mass spectrum was typical of normal long-chain aliphatic compounds, with a small peak at *m/e* 252 and a rather intense peak at *m/e* 192, the former corresponding to the M^+, and the latter to the $M^+ - 60$ of a tetradecadienyl acetate. Ozonolysis showed that the double bond nearer to the acetate group was in the 9-position. The NMR spectrum gave evidence [values for chemical shift (δ) in parts per million] for four olefinic protons (5.30), two methylene protons between two pairs of doubly bound carbon atoms (2.68) and a methyl group adjacent to a doubly bound carbon atom (1.65), along with those peaks associated with the acetate end of the molecule. The second double bond was therefore in the 12-position, and compound **II** is thus 9,12-tetradecadien-1-ol acetate with at least one of the double bonds possessing the *trans* configuration.



I

II

Comparison of the intensity of the band at 960 cm^{-1} relative to that of the band at 1030 cm^{-1} in the infrared spectrum of compound **II**, with the same relative intensity in the spectra of the four isomers of 5,9-tridecadien-1-ol acetate (*16*) showed that one double bond must be *cis* and the other *trans* in compound **II**. Synthesis of *cis*-9,*trans*-12-tetradecadien-1-ol acetate and comparison of its retention time by capillary DEGS gas chromatography (*17*), previously shown to separate the four geometric isomers of 5,9-tridecadien-1-ol acetate (*18*), with that of compound **II** showed them to be identical. Compound **II** is therefore *cis*-9, *trans*-12-tetradecadien-1-ol acetate.

Compound **I** has been synthesized previously (*15, 19*). Compound **II** was synthesized, in general, according to the procedure followed by Warthen and Jacobson (*16*) for the synthesis of *cis*-5,*trans*-9-tridecadien-1-ol acetate. Reaction of 1,8-octanediol with HCl gave 8-chloro-1-octanol (96 percent; boiling point, 86° to 88°C at 0.05 mm; n_D^{26} 1.4555), which was treated with 2,3-dihydropyran to form 2-[(8-chlorooctyl) oxy]tetrahydropyran (86 percent; boiling point, 114° to 117°C at 0.05 mm; n_D^{26} 1.4610). Coupling of this compound with lithium acetylide in dimethylsulfoxide gave 2-(9-decynyloxy) tetrahydropyran (74 percent; boiling point, 107° to 110°C at 0.2 mm; n_D^{26} 1.4597). Reduction of 2-butyn-1-ol with sodium in liquid ammonia gave *trans*-2-buten-1-ol (83 percent; boiling point, 115°C; n_D^{26} 1.4250), which was converted with phosphorus tribromide to *trans*-1-bromo-2-butene (66 percent; boiling point, 95° to 96°C; n_D^{26} 1.4760). Coupling of this compound with 2-(9-decynyloxy)-tetrahydropyran by means of lithium amide in dioxane gave tetrahydro-2-(*trans*-12-tetradecen-9-ynyloxy) pyran (29 percent; boiling point, 155° to 160°C at 0.08 mm n_D^{24} 1.4741), which was reduced by hydrogenation with Lindlar catalyst to tetrahydro-2-(*cis*-9,*trans*-12-tetradecadienyloxy)-pyran (86 percent; boiling point, 135° to 137°C at 0.04 mm; n_D^{24} 1.4680). Refluxing in acetic acid with acetyl chloride gave *cis*-9,*trans*-12-tetradecadien-1-ol acetate (91 percent; boiling point, 110° to 112°C at 0.04 mm; n_D^{18} 1.4572).

Spectral data for the corresponding isolated and synthesized compounds were identical, and the synthesized compounds were biologically active. We do not yet understand why the female southern armyworm moth produces two pheromones for the male moth, one of which is the natural sex pheromone of the fall armyworm moth. Perhaps compound **II** serves as a distance attractant for the male in nature, whereas compound **I** sexually excites the male when the sexes are in close proximity. The fact that compound **I** merely excites the male fall armyworm moth sexually without attracting it under natural conditions lends support to this theory.

MARTIN JACOBSON
ROBERT E. REDFERN
Entomology Research Division,
U.S. Department of Agriculture,
Beltsville, Maryland 20705
WILLIAM A. JONES*
MARY H. ALDRIDGE
Department of Chemistry,
American University,
Washington, D.C. 20016

References and Notes

1. C. L. Metcalf, W. P. Flint, R. L. Metcalf, *Destructive and Useful Insects* (McGraw-Hill, New York, 1962), p. 479.
2. Personal communication from B. A. Butt, USDA, Brownsville, Texas, in 1962.
3. R E. Redfern, *J. Econ. Entomol.* **60**, 308 (1967).
4. ——— and J. R. Raulston, *ibid.* **63**, 296 (1970).
5. B. A. Butt and E. Cantu, *U.S. Dep. Agr. Agr. Res. Serv. Bull. No. 33-75* (1962).
6. R. E. Redfern, B. A. Butt, E. Cantu, *J. Econ. Entomol.* **63**, 658 (1970).
7. R. W. Storherr, E. J. Murray, I. Klein, L. A. Rosenberg, *J. Ass. Offic. Agr. Chem.* **50**, 605 (1967).
8. Mesh 60 to 100, obtained from the Floridin Co., Tallahassee, Fla., and treated to contain 3 percent water. The mention of trade names or products does not constitute endorsement by the U.S. Department of Agriculture over those not named.
9. Adsorbosil-CABN, 60 to 100 mesh, containing 25 percent silver nitrate, obtained from Applied Science Laboratories, State College, Pa.
10. The chromatography was carried out on an F & M model 500 gas chromatograph with a stainless steel column packed with 5 percent OV-1 on 60 to 80 mesh GasChrom Q (3.05 m by 0.63 cm outside diameter). Column temperature was 200°C, and helium flow rate was 60 ml/min.
11. Stainless steel column (3.66 m by 0.63 cm outside diameter) packed with 5 percent stabilized DEGS on GasChrom Q (60 to 80 mesh). Column temperature was 175°C, and helium flow rate was 32 ml/min.
12. Mass spectra were obtained by means of an

LKB model 9000 gas chromatograph–mass spectrometer, equipped with a column (2.44 m by 0.63 cm outside diameter) of 1 percent SE-30 maintained at 150°C, with a helium flow rate of 20 ml/min.

13. Nuclear magnetic resonance spectra were obtained with a Varian HA-100 NMR spectrometer equipped with a C-1024 time-averaging computer.
14. M. Beroza and B. A. Bierl, *Anal. Chem.* **39**, 1131 (1967).
15. A. A. Sekul and A. N. Sparks, *J. Econ. Entomol.* **60**, 1270 (1967).
16. D. Warthen and M. Jacobson, *J. Med. Chem.* **10**, 1190 (1967).
17. The chromatography was carried out with a Varian Aerograph model 204-1B gas chromatograph having a stainless steel capillary column (91.5 m by 0.05 cm inner diameter) coated with DEGS. Column temperature was 175°C, and helium flow rate was 5.5 ml/min.
18. D. Warthen and N. Green, *J. Amer. Oil Chem. Soc.* **46**, 191 (1969).
19. D. Warthen, *J. Med. Chem.* **11**, 371 (1968).
20. Abstracted from a dissertation submitted to American University, Washington, D.C., by W.A.J., in partial fulfillment of the requirements for the Ph.D. degree.

21. We thank Mr. E. Cantu, U.S. Department of Agriculture, Brownsville, Texas, for supplying the insects and conducting the bioassays; Mr. E. L. Gooden, USDA, Beltsville, Md., for obtaining the NMR spectra; Dr. M. Thompson and Mr. R. C. Dutky, USDA, Beltsville, for obtaining the mass spectra; Dr. J. D. Warthen, USDA, Beltsville, for supplying a sample of synthetic compound **I** and for helpful suggestions; and Drs. W. R. Benson, Food and Drug Administration, Washington, D.C., and P. Andrulis, American University, Washington, D.C., for their helpful comments and suggestions.

* Present address: Entomology Research Division, U.S. Department of Agriculture, Beltsville, Md. 20705.

28 August 1970

Norepinephrine Metabolism in Brainstem of Spontaneously Hypertensive Rats

Abstract. *Concentrations of norepinephrine in lower brainstem and hypothalamus of genetically hypertensive rats are significantly lower than in control rats. There is a concomitant reduction (50 percent) in aromatic* L*-amino acid decarboxylase but not in tyrosine hydroxylase activity. A possible relation of this central catecholamine deficiency to the hypertension is discussed.*

Because catecholamines are neurohumoral pressor substances, it has long been suspected that they play a role in the pathogenesis of hypertension. However, generally speaking, investigators have been unsuccessful in demonstrating elevated concentrations of catecholamines in relation to hypertension. The spontaneously hypertensive rat (SHR) developed by Okamoto and Aoki (*1*, *2*) appears to be a suitable model of human essential hypertension and is now available as a genetically pure strain. Using this model we have investigated catecholamine metabolism in the central nervous system.

The blood pressures of male SHR's of F_{19-20} generations, control Wistar (NIH) and Sprague-Dawley rats (Zivic-Miller Lab. P.A.) from 5 to 20 weeks of age were measured without anesthesia by a tail plethysmographic method (*1*). Even in the earliest measurements (5 weeks after birth) the systolic blood pressure in SHR's was slightly higher than that in the controls. It was greatly elevated at 6 weeks of age and thereafter.

The SHR's and controls at 6, 10, and 20 weeks of age and the other rats in experimental groups at 20 weeks of age were killed by decapitation. Studies were done on whole brain (except for olfactory bulbs) and the following brain parts: lower brainstem (medulla oblongata, pons, and midbrain), brainstem (lower brainstem plus diencephalon), hypothalamus, and telencephalon. Various brain parts were dissected out, trimmed carefully, and immediately frozen for norepinephrine assay or chilled for enzyme assay. Mesenteric arteries from the root of superior mesenteric artery to the branches into the intestinal loop were dissected free from fat and connective tissue. The tissues were weighed and homogenized in cold 0.4*N* perchloric acid or in cold distilled water. Supernatant of the former, obtained after centrifugation, was analyzed for endogenous norepinephrine (*3*) and of the latter for aromatic L-amino acid decarboxylase activity (*4*) or for tyrosine hydroxylase activity (*5*). Protein concentration of the supernatant was determined by a modification of the phenol reagent method (*6*), and specific activities of these enzymes were calculated.

We reported recently that the concentration of norepinephrine was slightly, but probably not significantly ($P < 0.2$), decreased in the whole brainstem of the SHR's (*7*) as compared to control Wistar rats. However, looking specifically at the hypothalamus and lower brainstem there is a considerable reduction of norepinephrine in SHR's when compared to NIH control Wistar rats (Table 1). The average concentrations in 10-week-old SHR's in the initial stages of hypertension were about 60 and 70 percent of the control values in lower brainstem and hypothalamus, respectively. In these experiments the concentration of norepinephrine in the whole brain also appeared to be lower. This observation distinguishes this strain of SHR from the strain developed by Smirk and co-workers (*8*), who found their animals to have normal concentrations of norepinephrine in the brainstem but elevated concentrations in the cerebellum.

Aromatic L-amino acid decarboxylase activity in whole brainstem and telencephalon is clearly decreased in SHR's in comparison with that in normotensive control Wistar or Sprague-Dawley rats (Table 2). The enzyme activity in the SHR was about 50 percent of that in normotensive control Wistar or Sprague-Dawley rats. Even in the young, 6-week-old SHR, whose blood pressure was only slightly increased over the control level, the difference was clear-cut with no experimental point from SHR overlapping those of the controls. Animals with renal or deoxycorticosterone (DOC)-salt hypertension did not show any differences in the enzyme activity (Table 2). Consequently, this decrease in the activity did not appear to be due to secondary depression of enzyme activity by the hypertension itself. Since SHR's are considered to have slightly increased adrenocortical function (*2*, *9*), enzyme activity was also examined in adrenalectomized SHR's and control

Table 1. Norepinephrine content of the brain in spontaneously hypertensive and control rats. Values shown are means ± S.D. The numbers in parentheses indicate the number of animals used.

Group	Systolic blood pressure	Norepinephrine (μg/g)		
		Whole brain	Lower brainstem	Hypothalamus
		SHR		
10-weeks old (10)	176 ± 9*	0.268 ± 0.061†	0.453 ± 0.015*	1.220 ± 0.144*
20-weeks old (14)	214 ± 12*	0.235 ± 0.020*		
		Control Wistar (NIH)		
10-weeks old (10)	129 ± 4	0.333 ± 0.022	0.726 ± 0.072	1.725 ± 0.196
20-weeks old (14)	132 ± 5	0.323 ± 0.035		

* Significant difference from the control ($P < .001$). † Significant difference from the control ($.005 < P < .01$).