respect to the C(5′)-(S⁺) bond would be the so-called 'gauche, gauche', a conformation known to be predominant in free nucleosides[17].

Previous experiments using synthetic inhibitors[3] have shown that the terminal amino group of S-adenosyl homocysteine is important for binding to a t-RNA methyl transferase, whereas the carboxylate produces only a small contribution to this binding.

Careful observation of a molecular model of the proposed ternary complex suggests that the methyl group bound to sulphonium of SAM should point outwards from the enzyme in order the meet the t-RNA site of methylation. Preliminary experiments which will be reported later, have shown that chemical replacement of the methyl group in SAM by groupments such as allyl, methanoic or benzyl, led to compounds displaying affinity for a t-RNA methyl transferase from rabbit liver, comparable to SAM or close to the affinity displayed by SAM.

Finally, it would be of interest to check the validity of this base-pairing hypothesis by studying the sequences found in 'hypermethylated t-RNA'. This type of study was undertaken only in a few cases, therefore not allowing any statistical observations.

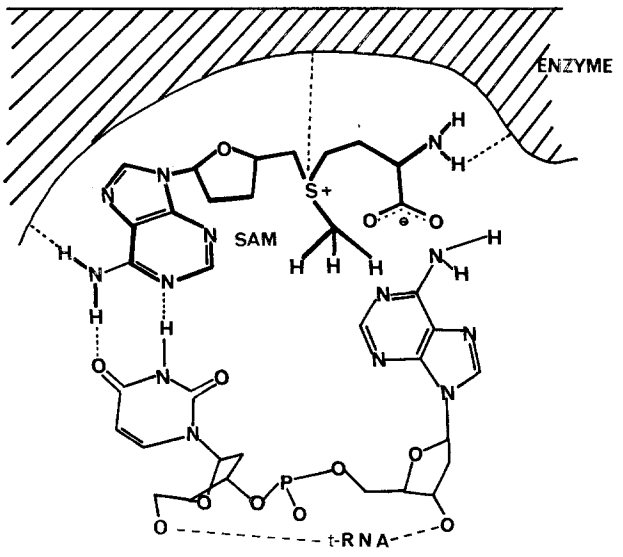

fig 6

Fig. 6. Hypothetical model of the interaction between a uridine of t-RNA and the S-adenosyl methionine-enzyme complex. The adenosyl methionine molecule is drawn with heavy lines. For the sake of clarity, neither the oxygen functions of ribose or phosphate are represented, nor the various hydrogen atoms attached to carbon or phosphorus.

Résumé. L'examen statistique des séquences des t-ARN indique qu'un nucléoside méthylé est généralement adjacent à une uridine ou à un analogue. Ces observations suggèrent que le processus de méthylation du t-ARN se ferait par l'intermédiaire d'un complexe ternaire dont les implications conformationnelles sont discutées.

J. HILDESHEIM, P. BLANCHARD and R. MICHELOT[18]

Institut de Chimie des Substances Naturelles, C.N.R.S., F–91190 Gif-sur-Yvette (France), 21 January 1974.

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[18] Acknowledgments. This work was supported by a grant from C.E.A. (Commissariat à l'Energie Atomique) for purchase of isotopes. We are grateful to Professor E. LEDERER for his constant interest and encouragement in this work and to Professor J. P. EBEL for helpful criticisms.

Cis-10-Tetradecenyl Acetate, an Attractant Component in the Sex Pheromone of the Oak Leaf Roller Moth (*Archips semiferanus* Walker)

The oak leaf roller, *Archips semiferanus* Walker (Lepidoptera: Tortricidae), is a destructive forest insect which has recently reached damaging population levels in the Northeastern United States. In Pennsylvania alone, the oak leaf roller infestation has spread to more than 1 million acres, and tree mortality due to the pest has climbed as high as 90%. The use of pesticides to control the oak leaf roller has not been economically feasible nor environmentally advisable; this has prompted our search for a natural chemical alternative.

Adult oak leaf roller males have been shown to respond to a sex pheromone produced by the female moth[1]. Moreover, preliminary separation of the active principles of the pheromone has revealed that two chromatographically isolable fractions are involved in the female's sexual message[2]. Each fraction elicits a separate behavioral response from male moths in laboratory and field assays, namely, sexual excitation and sexual attraction. We wish to report here on the identification, synthesis and activity of the major attractant.

Crude pheromone extracts were obtained from 2-day-old, adult, virgin female oak leaf rollers by excising the last abdominal segments and homogenizing them in a tissue grinder with redistilled spectrograde methylene chloride. The homogenate was filtered through a medium porosity fritted glass filter and concentrated on a rotary evaporator. Initial separation was achieved by thin layer chromatography on silica gel (Brinkmann Silplate F-22) using a 50:50 methylene chloride-hexane solvent system. An active region was observed at Rf 0.46–0.67 using a laboratory flight chamber bioassay[1]. The active TLC band was analyzed by gas-liquid chromatography (GC): 5% SE 30 on 80/100 mesh chromosorb Q; 6′, 2 mm I.D. glass U-tube column; oven, programmed from 125 °C to 200 °C at 1°/min.; helium carrier gas, 40 ml/min. The gas chromatograph was equipped with a flame ionization detector and an effluent splitter adjusted to a 25:75 detector:effluent split ratio. Small fractions were arbitrarily collected from the GC in glass capillary tubes cooled in liquid nitrogen. All fractions were laboratory and field bioassayed[1,2] and activity was found in only 1 fraction which had a retention time of 10.1 min. The

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[2] L. B. HENDRY, R. J. GILL, A. SANTORA and R. O. MUMMA, submitted (1974).

Number of oak leaf roller moths caught with various baits over a 5-day period in vane traps

Sample	♂	♀	Total	Net ♂ increase[a]
Cis-9-tetradecenyl acetate (500 ng)	3596	251	3847	580
Cis-10-tetradecenyl acetate (500 ng)	6426	289	6715	3410
Cis-11-tetradecenly acetate (500 ng)	3946	264	4210	930
Blank	3016	383	3399	—

[a] Number of oak leaf roller males trapped minus the number of males caught in the blank.

active SE 30 peak was then reinjected on a polar gas chromatographic column: 10% DEGS (Diethylene Glycol Succinate) on 80/100 mesh Chromosorb W; 6′, 2 mm I.D. glass U-tube column; oven, 135 °C isothermal; helium carrier gas, 35 ml/min. Several fractions were again collected and bioassayed. Only 1 fraction eluting at 21.0 min attracted males in the laboratory flight chamber. A second fraction, as yet unidentified, excited but did not attract males in the flight chamber[2]. The micro IR-spectrum of the attractive fraction was indicative of an unsaturated acetate: 5.79 μ, 8.11 μ ($OC-CH_3$), 6.12 μ (C = C). To determine its carbon skeleton, the fraction was then subjected to on-column hydrogenation[3] on a 10% DEGS glass U-tube column containing a pre column hydrogenator (1% palladium acetate on chromosorb P). A single peak was detected which had a retention time identical to that of an authentic sample of tetradecyl acetate indicating the active fraction contained an acetate having a 14 carbon chain.

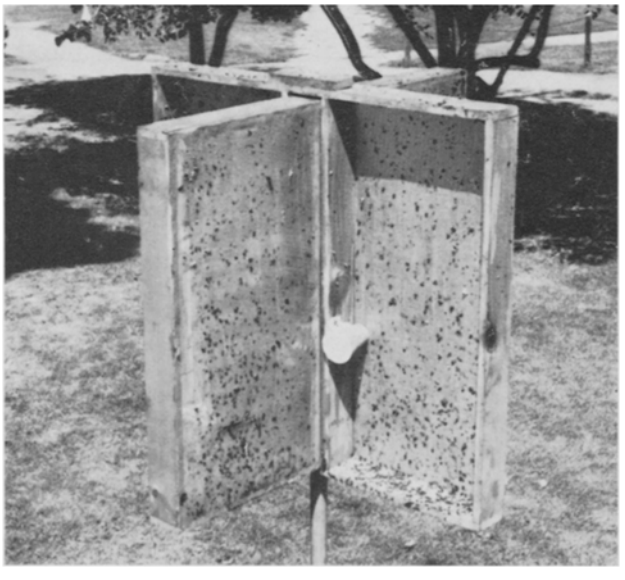
Micro ozonolysis[4] located the position of unsaturation in the chain. The active DEGS fraction was ozonized, and the products were analyzed on a 6′, 2 mm I.D. Porapak Q glass U-tube column at 150 °C (35 ml/min helium carrier gas). The retention times of the products were compared with those of standard aldehydes. A prominent peak which corresponded in retention time to butyraldehyde was observed; this required that a double bond be present in the 10 position. Moreover, the retention time of the active DEGS fraction fell between those of authentic samples of *cis*-9 and *cis*-11-tetradecenyl acetate[5] suggesting that the active principle was *cis*-10-tetradecenyl acetate (I).

$$CH_3CH_2CH_2\underset{cis}{CH=CH}(CH_2)_8CH_2OC(=O)-CH_3$$

I

To confirm our speculation, I was synthesized. Bromination of 1,10-decanediol in 48% HBr produced 10-bromodecanol in good yield.[6] Subsequent acetylation with acetyl chloride and oxidation using trimethylamine-N-oxide in chloroform yielded 10-acetoxy decanal[7]. Condensation of the aldehyde with the Wittig salt of bromobutane using sodium methoxide as a catalyst in DMF yielded *cis*-10-tetradecenyl acetate. The product was purified on a silica gel-silver nitrate column and shown to be better than 99% the *cis* isomer (silica gel-$AgNO_3$ TLC, GC). The spectral data for the *cis*-10-tetradecenyl acetate are: MS *m/e* 194 (M-HOAc), 166, 152, 138, 82 (base peak); and IR (CH_2Cl_2) 5.79 μ, 6.12 μ, 8.11 μ. Synthetic *cis*-10-tetradecenyl acetate had identical IR-, MS-, thin layer and gas chromatographic properties as the active DEGS GC fraction.

Studies were conducted to evaluate the attractiveness of *cis*-10-tetradecenyl acetate on oak roller males in the field. Large vane traps were constructed of a 90 cm square wood frame of 9 cm × 2 cm pine, covered on 1 side with window screen. The screen was attached in 2 sections with a gap in the center through which another slightly smaller frame could be inserted. The trap was supported 120 cm above the ground by a pole inserted through the center of the frame (see Figure), and the entire trap was coated with Tack Trap[8], a viscous sticky material suitable for catching and retaining insects. The traps were baited with various samples and set in areas of high oak leaf roller density immediately before the five day period that oak leaf roller males were flying in the field. The oak leaf roller moths which were trapped were sexed and counted. The data are compiled in the Table.



Vane trap used to test attraction of oak leaf roller males to various baits in the field.

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[6] F. L. M. Pattison, J. B. Stothers and R. G. Woolford, J. Am. chem. Soc. *78*, 2255 (1956).
[7] V. Franzen and S. Otto, Chem. Ber. *94*, 1360 (1961).
[8] Animal Repellents, Inc., Griffin, Ga., USA.

Oak leaf roller males were caught in all traps including those baited with known sex attractants of other moths, i.e. *cis*-9-tetradecenyl acetate[9] and *cis*-11-tetradecenyl acetate[10]. However, synthetic *cis*-10-tetradecenyl acetate caught 3 to 6 times more oak leaf roller males than either of the known attractants after subtracting the number of males trapped in the blank. The sizable number of males caught in the blank was expected due to the large numbers of males randomly flying in the test areas, and the attraction of males by pheromone producing females which had been caught in the traps.

In summary, *cis*-10-tetradecenyl acetate is a major component of the chemical message which attracts oak leaf roller males to their mates. To our knowledge, this is the first reported identification of this compound in an insect. Large scale synthesis of this attractant will enable extensive field testing and subsequent analysis of its potential as an effective control for the oak leaf roller. Further chemical analyses of the female extracts for additional chemical messengers including sexual excitants are being conducted[11].

Zusammenfassung. Weibchen der Oak Leaf Roller, *Archips semiferanus* Walker, benutzen ein Geschlechtspheromon, um die Männchen der Art zur Paarung zu locken. Ein Lockstoffbestandteil im Pheromon wurde von den Bauchextrakten der Weibchen isoliert und als *cis*-10-Tetradecenyl-Acetat, eine in Insekten bisher unbekannte Verbindung, identifiziert. Fangversuche im Freien mit dem synthetischen Pheromon bestätigten seine Rolle als Lockstoff.

L. B. HENDRY, J. JUGOVICH, L. ROMAN, M. E. ANDERSON and R. O. MUMMA

Departments of Chemistry and Entomology, The Pennsylvania State University, 152 Davey Laboratory, University Park (Pennsylvania 16802, USA),
15 February 1974.

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[10] W. L. ROELOFS and H. ARN, Nature, Lond. *219*, 513 (1968).
[11] This work was partially supported by a grant from Research Corporation (RC-inhibitor No. 3733). Authorized for publication as paper No. 4631 in the Journal Series of The Pennsylvania Agriculture Experiment Station.

Hypogravity-Induced Inhibition of CO_2 Production from Amino Acids in Higher Plants

The effect of hypogravity on the growth and physiology of higher plants has been simulated by means of the horizontal rotation of the plant on a clinostat[1]. The appearance of leaf epinasty has been the only criterion utilized for determining when hypogravity has affected the normal physiology of the plant. We have been investigating the effect of simulated hypogravity on the metabolism of certain amino acids in higher plants. L-proline-U-^{14}C was infiltrated by use of a wick[2] into 50-day-old marigold plants (*Tagetes patula*) mounted on vertical and horizontal clinostats rotating at 4 rph (revolutions per h). Normal plants were similarly infiltrated. After 24 h of incubation the various tissues of the plants were extracted with aqueous ethanol and the free amino acid fraction examined by two-dimensional paper chromatography and radioautography. A major radioactive constituent in every case was γ-aminobutyric acid. Since the conversion of proline to glutamate is a well-known route of proline metabolism[3], the presence of an active glutamate decarboxylase was indicated.

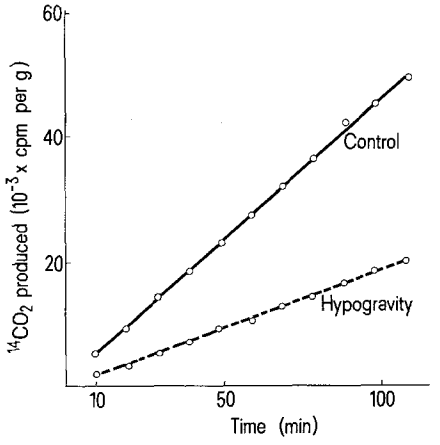

Fig. 1. The production of $^{14}C_2O$ from control plants compared to those rotated on horizontal clinostats after infiltration of 5 µCi of L-glutamic acid-U-^{14}C (specific activity 175 mCi per mmole). 42-day-old plants were rotated on the clinostats for 4 days before infiltration. The results are given on a fresh weight basis.

The in vivo conversion of L-proline-U-^{14}C to $^{14}CO_2$ in normal and hypogravity plants under continuous illumination was then measured and compared with plants similarly treated with L-glutamic-U-^{14}C and others with L-valine-U-^{14}C. Marigold plants (var. Petite Gold), between 30 to 50 days old, were mounted on horizontal clinostats rotating at a speed of 15 rph and left for at least 4 days. Control plants were rotated on vertical clinostats. The labelled amino acid was injected into the stem of the plant at the desired time by means of a syringe. Immediately after the injection the plants were placed in clinostat gas exchange chambers mounted on the clinostats. The chambers were swept with compressed air from a cylinder. The air passing over the plant was bubbled through 5 ml of a 1 *M* solution of hyamine hydroxide so as to trap the released $^{14}CO_2$. The trapping solution was replaced every 10 min with fresh hyamine. Aliquots of the hyamine solutions were mixed in 10 ml Bray's solution[4] and then assayed for radioactivity in a liquid scintillation counter. At the conclusion of the experiment the plants were cut just above the point of label application and weighed.

The results of Figures 1 and 2 demonstrate very clearly that $^{14}CO_2$ production is much higher in the control plants than in those subjected to hypogravity. Since

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