Materials	Irradiation time, min.	% Up	% Cp	% Ap	% Gp	% X [100−(Up +Cp+Ap +Gp)]
RNA	0	24.9	22.3	25.0	27.7	0
(Baker's yeast)	60	14.3	21.8	25.1	27.5	11.3
	120	9.0	21.4	25.2	27.5	16.9
	180	6.5	20.5	25.0	27.4	20.6
	240	5.6	21.1	25.3	27.3	20.7
Calf-liver transfer RNA	0	18.1	26.6	17.6	37.7	0
	270	10.4	26.3	17.0	36.9	9.4
E. coli B. transfer RNA	0	19.4	27.0	20.2	33.4	0
	270	11.7	25.8	20.1	33.7	8.7

[a] Baker's yeast according to Crestfield, et al., Sigma.

For the sequence determination of polynucleotides the dihydrouridine units offer a handle for changing the pattern of enzymatic cleavage. The reductive modification of uridine-containing nucleotides, including coding triplets, may now help to answer a number of questions in biogenetics.

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Biosynthesis of Arthropod Secretions.
I. Monoterpene Synthesis in an Ant
(Acanthomyops claviger)[1,2]

Sir:

We wish to report the first experimental evidence for monoterpene synthesis in an arthropod. It is well known that many arthropods are able to accumulate repellent defensive secretions.[3] These secretions may contain from one to a large number of relatively simple components, including aliphatic hydrocarbons, carbonyl compounds, carboxylic acids, terpenes, phenols, and quinones.[3] In spite of extensive biological and chemical studies of this group of natural products, little is known of their origin. The possibility that insects and other arthropods which possess these secretions acquire and concentrate the preformed toxic components from their diet has been supported recently by Brower and Brower.[4] On the other hand, preliminary investigations of Gordon, Waterhouse, and Gilby indicate that green vegetable bugs [Nezara viridula (Fabr.) Pentatomidae] are able to incorporate acetate into a very complex mixture of aliphatic compounds.[5]

(1) Partial support of this work by the National Institutes of Health (Grant AI 2908) is acknowledged with pleasure.

(2) Presented in part at the Symposium on Bio-Organic Chemistry, University of California at Santa Barbara, Jan. 21, 1965, and at the 149th National Meeting of the American Chemical Society, April 1965, Detroit, Mich.

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We have chosen for initial study the terpenoid secretion of the mandibular glands of an ant [Acanthomyops claviger (Roger)]; the chief components of this secretion have recently been identified as citronellal (I) and citral (II), in a ratio of about 9:1.[6] Not only are these terpenes of defensive importance, but, along with a number of other isoprenoid substances, they also take on a role in chemical communication,[7] which makes this group of compounds especially significant.

CHO   CHO

I   II
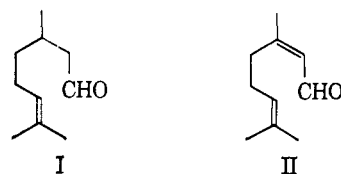
Groups of 1000–1500 worker ants were freshly collected near Ithaca, N. Y., and maintained in laboratory colonies for the duration of each experiment. In separate experiments, these ants were fed portions of the potential precursors (sodium 1-^{14}C-acetate, sodium 2-^{14}C-acetate, 2-^{14}C-mevalonic lactone) in aqueous glucose solutions. After 7–10 days, the ants were frozen and extracted with methylene chloride. A few milligrams of cold I and II were added to the extract as carrier. Thin layer chromatography [silica gel G developed with hexane–ethyl acetate (92:8)] separated I and II from each other and from the other lipids. The two aldehydes were detected by spraying the plates with a solution of 2,4-dinitrophenylhydrazine in tetrahydrofuran.[8] The eluted terpenes were further treated with the same reagent in order to complete the formation of their 2,4-dinitrophenylhydrazones. The separated derivatives were subjected to four successive thin layer chromatographic purifications [silica gel G (benzene), aluminum oxide G (petroleum ether–diethyl ether, 24:1), silica gel G (chloroform), and silica gel G (diethyl ether)] and brought to constant specific activity. The results of these experiments are summarized in Table I.[9]

Table I

Compounds fed to A. claviger			Activity of terpene aldehyde, d.p.m./mmole	
Compd.	Amount, mg.	Activity, d.p.m.	Citronellal	Citral
Acetate-1-[14]C	0.18	2×10^7	3.3×10^6	3.3×10^6
Acetate-2-[14]C	0.048	3×10^7	3.3×10^6	2.7×10^6
Mevalonate-2-[14]C	2.32	1×10^7	1.4×10^6	1×10^6
Mevalonate-1-[14]C	6.2	1×10^7	0	0

It is apparent from these results that these ants are able to use the labeled acetate and mevalonate for terpene biosynthesis, and that they need not rely on preformed terpenes in their food. The utilization of these specific precursors further suggests that the normal "mevalonic acid" pathway of terpene biosynthesis is
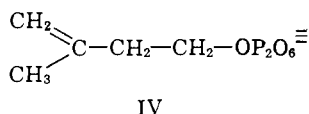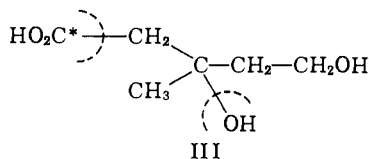
(6) M. S. Chadha, T. Eisner, A. Monro, and J. Meinwald, J. Insect Physiol., 8, 175 (1962).

(7) For a general review of the importance of isoprenoid compounds in arthropods, see H. A. Schneiderman and L. I. Gilbert, Science, 143, 325 (1964).

(8) H. J. Shine, J. Org. Chem., 24, 252, 1790 (1959).

(9) The per cent incorporation obtained in these experiments ranged from 0.07 to 0.10% for citronellal.

being utilized.[10] For additional information on this point, a feeding experiment was carried out using 1-^{14}C-mevalonate (III). Here, the results (Table I)

$$HO_2C*\text{---}CH_2$$
$$\underset{CH_3}{\diagup}C\text{---}CH_2\text{---}CH_2OH$$
$$OH$$

III

$$\underset{CH_3}{\overset{CH_2}{\diagdown}}C\text{---}CH_2\text{---}CH_2\text{---}OP_2O_6^{\equiv}$$

IV

showed negligible ^{14}C incorporation, as would be expected if III were converted into Δ^3-isopentenyl pyrophosphate (IV), with concomitant loss of ^{14}C as carbon dioxide prior to terpene formation.[10]

Birch, et al., have studied the incorporation of 2-^{14}C-acetate into citronellal in Eucalyptus citrodora (Hook) and found extensive randomization of the carbon atoms, tentatively attributed to the formation of ^{14}CO$_2$, followed by photosynthetic reincorporation of the ^{14}C.[11] It will be interesting to investigate the pattern of isotopic incorporation for the animal case. We hope to describe these and related results in more detail in subsequent publications.

(10) An excellent recent review of the very extensive literature in this field is provided by J. H. Richards and J. B. Hendrickson, "The Biosynthesis of Steroids, Terpenes and Acetogenins," W. A. Benjamin, Inc., New York, N. Y., 1964.
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(12) National Institutes of Health Postdoctoral Fellow, 1964–1965.

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Application of Taft's Fluorine-19 Chemical Shift Correlations to the Study of Secondary Deuterium Isotope Effects[1]

Sir:

The subject of secondary deuterium isotope effects has been of considerable interest in recent years in regard to their origin and their application to studies of mechanisms.[2-4] While it is generally understood that the origin of these effects is related to differences in vibrational amplitudes or zero point energies, interpretations have been put forth based on an apparently greater inductive electron-donating tendency of –C–D than –C–H, and a reduced hyperconjugative donating ability of –C–D compared to –C–H.

To investigate the usefulness of discussing these essentially vibrational phenomena in terms of the normal substituent characteristics of inductive and resonance effects we have attempted to detect isotope effects on the separate inductive and resonance σ-constants, σ_I and $\sigma_R°$, of the methyl group by utilizing Taft's correlations of these constants with the F^{19}

(1) Reproduction in whole or in part is permitted for any purpose of the U. S. Government.
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chemical shifts of meta- and para-substituted fluorobenzenes.[5-9] These correlations which are based on experimental work[5-9] as well as theoretical justification[10,11] are presented in the following equations, where $\int_H^{p-x}$ and $\int_H^{m-x}$ are the fluorine chemical shifts in p.p.m. of para- and meta-substituted fluorobenzene, respectively, relative to a fluorobenzene standard.

$$\int_H^{p-x} - \int_H^{m-x} = (-29.5)\sigma_R° \tag{1}$$

$$\int_H^{m-x} = -7.1\sigma_I + 0.60 \tag{2}$$

Although measured secondary deuterium isotope effects invariably have been small, the sensitivity of the F^{19} method seemed to qualify it for such a study. We therefore prepared p- and m-fluorotoluene-α,α,α-d_3 and determined their F^{19} chemical shifts with respect to their undeuterated analogs.

The deuterated compounds were prepared[12] from the corresponding methyl fluorobenzoates, via lithium aluminum deuteride reduction to the fluorobenzyl-α,α-d_2 alcohols, conversion with thionyl chloride to the α-chlorofluorotoluene-α,α-d_2 compounds, and decomposition of the corresponding Grignard reagents with deuterium oxide. Fluorine-19 magnetic resonance measurements were made at 56.4 Mc.p.s. on the pure fluorotoluenes, mixtures of deuterated and nondeuterated analogs, and solutions in carbon tetrachloride.

The F^{19} spectra of m-fluorotoluene, m-fluorotoluene-α,α,α-d_3, and a 1:1 mixture of the two compounds gave nearly identical patterns of precisely defined peaks, indicating no chemical shift difference within experimental limits and uncertainties of about ± 0.2 c.p.s. This result would seem to imply, in terms of eq. 2, that there is no difference between the inductive constants σ_I for the groups CH$_3$ and CD$_3$, at least within the framework of Taft's F^{19} correlation technique. This is in contrast to conclusions based on chemical techniques,[2,3] from which a F^{19} shift difference as high as 4 c.p.s. might have been predicted.[12]

The F^{19} spectra of a 2:1 mixture of p-fluorotoluene and p-fluorotoluene-α,α,α-d_3 and of a 1:1:1 (volume) mixture of these components with carbon tetrachloride gave complicated patterns of overlapping peaks. These patterns are consistent with the superposition of the spectra of the components if it is assumed that the chemical shift of the undeuterated compound is about 0.7 ± 0.2 c.p.s. greater than that of the deuterated analog.[12] In terms of eq. 1 this corresponds to a difference of 4×10^{-4} between the values of $\sigma_R°$ for CD$_3$ and CH$_3$ (CD$_3$ having the more positive value). The 0.7 c.p.s. shift is in the direction to be expected

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(12) Details of these experiments and a more complete discussion of the results will be presented elsewhere.