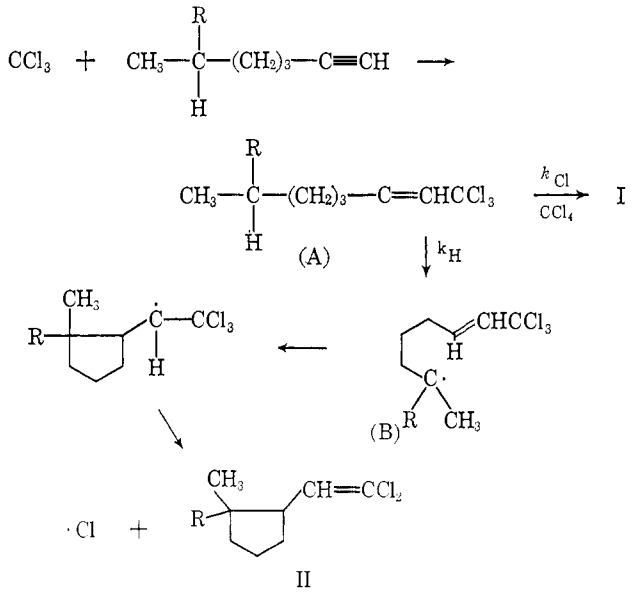
band at 1618 cm^{-1}, indicative of a $-C{=}CCl_2$ linkage.[8] *Anal.* (of the isomer mixture): Calcd for $C_8H_{12}Cl_2$: C, 53.63; H, 6.76; Cl, 39.6. Found: C, 53.33; H, 6.87; Cl, 39.1.

The skeletal structure of the rearranged product II was confirmed by quantitative hydrogenation of the isomer mixture, in the presence of Raney nickel and alcoholic KOH,[9] to a mixture of *cis*- and *trans*-1-methyl-2-ethylcyclopentane, with the *cis* isomer predominating. The vpc retention times of these products as well as their mass spectra were identical with those of authentic samples.

The other major product, III, bp 45–50° (8 mm), was shown to be the Cl_2 addition product of 1-heptyne. Its nmr spectrum and vpc retention time were identical with those of an authentic sample.

When 6-methyl-1-heptyne was used in place of 1-heptyne, the ratio of rearranged cyclic product over the normal 1:1 adduct (R:N) increased to more than 6:1, based on nmr analysis of the crude reaction product as well as on actual isolated yields. The rearranged product, bp 86–89° (10 mm), consisted of a single isomer. *Anal.* Calcd for $C_9H_{14}Cl_2$: C, 55.95; H, 7.31; Cl, 36.7. Found: C, 55.90; H, 7.32; Cl, 36.6. Its nmr spectrum contained a doublet at τ 4.3 (J = 9.7 cps, 1 H) as well as two peaks at τ 9.0 (3 H) and 9.2 (3 H), in addition to broad absorption in the τ 7.3–8.8 region.

The formation of compound II as a major product can best be explained by the free-radical chain mechanism



This mechanism displays three reaction steps involving free radicals: first, intramolecular 1,5-hydrogen abstraction, followed by internal cyclization *via* addition to a double bond,[10] and finally halogen elimination from a β-halo radical.[11] This halogen elimination is considerably faster than Cl transfer from CCl_4 in our system.

The ratio of R:N obtained with 1-heptyne increased with increasing reaction temperature. The activation energy difference was found to be $E_H - E_{Cl} = 2.7 \pm 0.5$ kcal/mole with $\log A_H/A_{Cl} = 1.4 \pm 0.3$.

The following points can be deduced from our results: (1) the low ratio of the frequency factors (A_H/A_{Cl}) is in accord with a cyclic six-membered transition state in which the hydrogen from C_5 is abstracted in preference to any other hydrogen; (2) the observed 1,5-hydrogen shift in vinyl radicals, while completely absent from the corresponding secondary alkyl radicals, must be due to the greater reactivity of vinyl radicals which is in accord with the relatively high vinyl carbon–hydrogen dissociation energy.[12] With secondary alkyl radicals the activation energy difference ($E_H - E_{Cl}$) would be expected to be greater than 2.7 kcal/mole and hence the rate of chlorine transfer from CCl_4 would greatly exceed that of internal 1,5-hydrogen shift.

(8) R. Dowbenko, *Tetrahedron*, **21**, 1647 (1965).
(9) L. Horner, L. Schlafer, and H. Kammerer, *Ber.*, **92**, 1700 (1959).
(10) C. Walling and M. S. Pearson, *J. Am. Chem. Soc.*, **86**, 2262 (1964); P. W. Ayers, *J. Org. Chem.*, **30**, 3099 (1965), and references therein.
(11) C. Walling, "Free Radicals in Solution," John Wiley and Sons, Inc., New York, N. Y., 1957, p 302.
(12) (a) Reference 11, p 50; (b) A. G. Harrison and F. P. Lossing, *J. Am. Chem. Soc.*, **82**, 519 (1960).

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Biosynthesis of Arthropod Secretions. III. Synthesis of Simple *p*-Benzoquinones in a Beetle (*Eleodes longicollis*)[1]

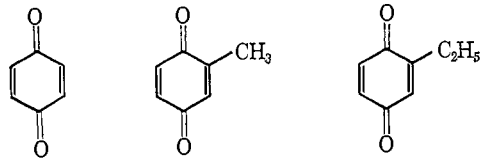
Sir:

While the composition of arthropod defensive secretions has been the subject of much investigation,[2] little attention has been given to the origin of their repellent components. We have recently shown that two monoterpenes which play a defensive role among insects are synthesized from acetate.[3] Simple *p*-benzoquinones are also widespread components of such defensive secretions and we now wish to report that these quinones appear to arise *via two independent pathways*. The first of these involves utilization of the preformed aromatic ring of tyrosine or phenylalanine, a mechanism which finds analogy in ubiquinone biosynthesis in animals.[4] The second involves building up of the quinone ring from acetate units, a well-known microbiological mechanism for aromatic ring biosynthesis,[5] only recently considered to occur in animals.[4,6]

For the initial study of the biosynthesis of *p*-benzoquinones in arthropods, we chose to investigate the

(1) Presented in part at the Third International Symposium on the Chemistry of Natural Products, Kyoto, Japan, April 1964 Abstracts, p 138.
(2) L. M. Roth and T. Eisner, *Ann. Rev. Entomol.*, 7, 107 (1962); H. Schildknecht, *Angew. Chem.*, **75**, 762 (1963).
(3) G. Happ and J. Meinwald, *J. Am. Chem. Soc.*, **87**, 2507 (1965); J. Meinwald, G. Happ, J. Labows, and T. Eisner, *Science*, **151**, 79 (1966).
(4) J. Glover in "Biochemistry of Quinones," R. A. Morton, Ed., Academic Press Inc., New York, N. Y., 1965, Chapter 7.
(5) A. J. Birch in "Ciba Foundation Symposium, Quinones in Electron Transport," Little, Brown and Co., Boston, Mass., 1962, p 233.
(6) For recent evidence of aromatic biosynthesis from aliphatic precursors in a nematode worm (*Caenorhabditis briegsae*) and in a female cockroach (*Periplaneta americana*), see M. Rothstein and G. Tomlison, *Biochim. Biophys. Acta*, **63**, 471 (1962); P. C. J. Brunet, *Nature*, **199**, 492 (1963). Since our experiments were carried out with entire, intact beetles, the results obtained apply to this animal *including* any symbiotic organisms to which the beetle is normally a host, a limitation also applicable to the above cited study of *P. americana*.

defensive secretion of a tenebrionid beetle, *Eleodes longicollis*.[7] From this secretion *p*-benzoquinone (I), methyl-*p*-benzoquinone (II), and ethyl-*p*-benzoquinone (III) can easily be isolated and purified.



I II III

In an exploratory screening program, a variety of potential precursors were administered to small groups of beetles by feeding or injection. After a 4-day period the secretion was collected and treated with 2,4-dinitrophenylhydrazine. The resultant derivatives were separated and purified by paper chromatography. Among the compounds studied were ^{14}C-labeled tyrosine, phenylalanine, sodium acetate, sodium propionate, and sodium malonate. The results are summarized in Table I.

Table I

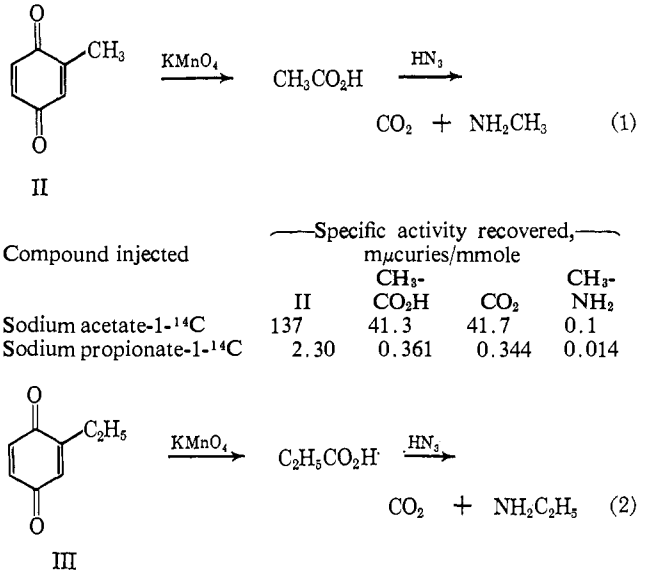
Compound	Activity administered, dpm	Activity recovered, dpm[a] I	II	III
DL-Tyrosine-2-^{14}C[b]	1×10^7	0	5×10^2	2×10^2
L-Tyrosine-U-^{14}C[b]	1×10^7	2×10^4	2×10^2	2×10^2
DL-Phenylalanine ring-^{14}C[b]	1×10^7	3×10^4	2×10^2	1×10^2
Sodium acetate-2-^{14}C[c]	2×10^8	4×10^3	2×10^5	1×10^5
Sodium propionate-2-^{14}C[c]	1×10^8	1×10^3	4×10^4	1×10^5
Sodium malonate-2-^{14}C[c]	9×10^7	2×10^3	2×10^5	4×10^5

[a] These results are given in disintegrations per minute per sample (1–6 mg) of 2,4-dinitrophenylhydrazine derivative. [b] Fed. [c] Injected.

From these results it is apparent that while the main pathway for the synthesis of *p*-benzoquinone (I) involves the utilization of the aromatic ring of tyrosine or phenylalanine, this is not the case for the alkylated quinones II and III. The data for the incorporation of sodium acetate and sodium propionate into II and III can be accommodated by the acetate hypothesis or by an alternative hypothesis in which these aliphatic precursors provide a side chain which is then coupled to an aromatic ring of unknown origin.[8] However, the data showing extensive incorporation of sodium malonate support the former hypothesis, which normally involves the condensation of malonate units with an acyl coenzyme A.[5]

In further experiments aimed at defining the patterns of incorporation into II and III, sodium acetate-1-^{14}C and sodium propionate-1-^{14}C were injected into the insects. The defensive secretions were collected over a 2-week period and then directly separated into their component *p*-benzoquinones by column chromatography on silicic acid, without preliminary formation of a derivative. Active quinones II and III, containing about 0.01 to 0.1% of the carbon-14 administered, were diluted with carriers and oxidized to acetic acid and propionic acid by potassium permanganate. These aliphatic acids were then degraded by means of the Schmidt reaction[9] (eq 1 and 2). The products were purified to constant specific activity and gave the results summarized in Scheme I.[10]

Scheme I[a]



II

Compound injected	Specific activity recovered, mμcuries/mmole II	CH_3-CO_2H	CO_2	CH_3-NH_2
Sodium acetate-1-^{14}C	137	41.3	41.7	0.1
Sodium propionate-1-^{14}C	2.30	0.361	0.344	0.014

III

Compound injected	Specific activity recovered, mμcuries/mmole III	CH_3CH_2-CO_2H	CO_2	CH_3CH_2-NH_2
Sodium acetate-1-^{14}C	36.4	3.82	3.60	0.28
Sodium propionate-1-^{14}C	25.0	23.5	23.0	0.3

[a] Specific activity of diluted samples. These activities were determined by combustion of the samples and direct counting of the resultant carbon dioxide.

It is interesting to note that about 30% of the total activity of II derived from sodium acetate-1-^{14}C appears at C_2. Since less than 0.1% of the activity occurs in the methyl group of II, *the remaining 70% must be distributed among the other five ring carbon atoms.* Similarly it may be observed that in the case of III derived from sodium acetate-1-^{14}C, about 90% of the total activity is found in the analogous five ring carbons. The ability of acetate-derived units to serve as building blocks for the six-membered rings of the beetle quinones II and III is thus established.

The most significant result which arises from the sodium propionate-1-^{14}C incorporation experiments is that about 95% of the activity found in III is recovered in the derived propionic acid, and is again almost entirely localized at C_2 of the ring. The low level of incorporation of this precursor into the remaining ring carbons of III and into II is easily rationalized by slight conversion of this precursor into two-carbon units. Thus, condensation of propionyl coenzyme A with malonate units and ultimate cyclization would accommodate these distributions readily.

The significance to the beetle of maintaining a pathway leading to I which is independent of that leading

(7) M. S. Chadha, T. Eisner, and J. Meinwald, *J. Insect Physiol.*, 7, 46 (1961).

(8) M. Pavan, *Intern. Congr. Biochem., 4th, Vienna, 1958*, 12, 15 (1959).

(9) The technique used was that of R. C. Anderson and A. P. Wolf, Brookhaven National Laboratory Report 3222, New York, N. Y. This is a modification of the method of E. F. Phares, *Arch. Biochem. Biophys.*, 33, 173 (1951).

(10) For a description of the assay method, see D. R. Christman, N. E. Day, P. R. Hansell, and R. C. Anderson, *Anal. Chem.*, 27, 1935. (1955).

to its alkylated homologs II and III is not obvious. It is hoped that subsequent work on benzoquinone biosynthesis in other arthropods may clarify this problem.

Acknowledgment. We are indebted to Dr. Alfred P. Wolf of the Chemistry Department of the Brookhaven National Laboratories for providing valuable facilities during part of this work. We also wish to thank Dr. Wolf and Miss Carol S. Redvanly for helpful discussions regarding the degradation of the quinones, and Dr. D. R. Christman and Catherine T. Paul who performed the carbon-14 assays.

(11) On leave from Wellesley College, Wellesley, Mass. This investigation was supported in part by Public Health Service Special Postdoctoral Fellowship 1-F3-GM-10, 441-01 from the National Institute of General Medical Sciences, and in part by Research Grant AI-02908 from the U. S. Public Health Service.

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Book Reviews

Advances in Chemotherapy. Volume 1. Edited by ABRAHAM GOLDIN, National Cancer Institute, National Institutes of Health, U. S. Public Health Service, Bethesda, Md., and F. HAWKING, National Institute for Medical Research, Mill Hill, London, England. Academic Press Inc., 111 Fifth Ave., New York, N. Y. 1964. xi + 579 pp. 15.5 × 23.5 cm. $17.50.

This book represents a remarkable achievement in fulfilling the goals which its editors had set for themselves: "to offer a common meeting ground for investigators in chemotherapy who may have come into this subject from organic chemistry, biochemistry, pharmacology, genetics, microbiology, parasitology, immunology, physiology, pathology, or other scientific disciplines." It accomplishes an excellent balance between theory, fundamental principles, and experimental findings in the field of chemotherapy which should appeal both to the experts, as well as to the "novices" in this area of drug therapy. It offers a dynamic view of drug interaction at the cellular level both in the host and the invading microorganism without sacrificing on the details of experimental evidence. While speculative in many instances as to the mode of action of chemotherapeutic agents, the authors, nevertheless, treat their own speculations with an air of detachment and a healthy amount of skepticism.

The introductory chapter by E. K. Marshall on "Historical Perspectives in Chemotherapy" sets the stage for this valuable treatise and brings the reader quickly up-to-date on past accomplishments in the rapidly growing areas of the chemical treatment of parasitic, viral, and neoplastic diseases. While this book is written by nine different authors, it does achieve a measure of cohesiveness seldom seen in works of this nature.

It is noteworthy that at the very outset the reader is apprized of the difficulties which beset the clinical investigator in selecting a candidate agent for clinical evaluation on the basis of biochemical, animal pharmacologic, and toxicologic data which may or may not be predictive of the ultimate activity and toxicity of the new drug in man. While this chapter by Zubrod on the "Quantitative Concepts in the Clinical Study of Drugs" is, of course, applicable to all types of therapeutic agents, it does point to the particular plight of the clinician dealing with the treatment of neoplastic diseases where drugs have to be administered at near toxic levels to produce a regression of the disease. The plea made by the author for clinical relevancy of toxicologic, biochemical, and pharmacologic test procedures is well taken, since all too often, animal methodology is developed with little attempt to simulating actual clinical conditions. The interdisciplinary approach to phases I and II in the clinical evaluation of a candidate agent receives particular emphasis. Continued surveillance of the new drug for several years once it has been "turned loose on the community" is strongly advocated by the author to establish an epidemiological pattern of side effects and toxicities in a given population.

Newton's chapter on the "Mechanism of Action of Phenanthridine and Aminoquinaldine Trypanocides" points to the complexities of combating trypanosomiasis which is influenced by climate, altitude, vegetation, and pattern of human migration. The disadvantages of the *Crithidia oncopelti* test system are discussed in the light of the differences of the drug effects in insect forms as compared to those obtained in the blood stream.

While little is known relative to the biochemistry and mechanism of action of the quinaldines and phenanthridines, the interaction of quinapyramine and homidium with various cellular systems is discussed. Quinpyramine changes the growth pattern of the trypanosome cell system from an exponential to a linear form. Hence, while this group of drugs is not trypanocidal, it reduces the trypanosome population sufficiently for the body's defenses to take over. The use of washed cell suspensions to study the effects of these drugs on nucleic acid and protein synthesis reveals that quinapyramine causes a progressive inhibition of RNA synthesis which may be reversed by *p*-aminobenzoic acid. The trypanocidal drug, homidium, on the other hand, inhibits the synthesis of DNA in the trypanosome cell. Failure of quinapyramine to inhibit DNA synthesis and glycine incorporation into nucleic acid is thought to be due to the inability of the drug to penetrate the cell nucleus. Evidence is presented which indicates that quinapyramine modifies the normal binding of ribosomal RNA to protein. The aggregation of ribosomes caused by the presence of the drug results in loss of their biological activity. C^{14} adenine incorporation into nucleic acids is inhibited by quinapyramine.

The effect of homidium and quinapyramine on other cell systems is discussed; *e.g.*, in Ehrlich's ascites tumors and *E. coli*, homidium is a potent inhibitor of purine incorporation into nucleic acids.

Speculations to explain the phenomenon of selective toxicity (damage to parasitic cell, but not to host cell) of these two groups of drugs are offered by the author.

Studies of the high degree of cross resistance between the various curative and prophylactic agents are complicated by the fact that it is difficult to obtain resistant strains in laboratory animals.

In bacteria, homidium is a potent inhibitor of protein and nucleic acid synthesis. Several of the phenanthridines also inhibit the development of the influenza virus in eggs.

The chapter by R. G. Thompson on the "Chemoprophylaxis and Chemotherapy of Viral Diseases" is a concise summary review of the various chemical structural types that have displayed antiviral activity *in vitro* and in intact animals. Of all these drug types, only 2′-dioxy-5-iodouridine (IUDR) has become commercially available for the treatment of keratitis produced by *Herpes simplex* and *vaccinia* virus. Both viruses contain only DNA; IUDR interferes with the synthesis of DNA and thereby inhibits replication. It has no activity on RNA containing viruses. Thompson points out that there are either DNA or RNA containing viruses and drugs normally active against one type of virus will usually be inactive on the other virus strain.

The role of interferon, an endogenous substance which is released by the body during viral infections, is discussed with respect to its action as a prophylactic agent and an inhibitor of virus production. It acts against both RNA and DNA containing viruses.

Two other drugs, cytosine arabinoside and isatin β-thiosemicarbazone, also display promising antiviral properties.

While at present, there can be little correlation between chemical structure and antiviral activity, the fact that a few of the synthetic