quantitatively the relative reactivities of various classes of olefins toward methyl radical additions. The respective relative rate constants of the addition, frequently referred to as methyl affinities, are determined as ratios k_2/k_1, where the subscripts refer to the two reactions

$$CH_3 + \text{isoöctane} \longrightarrow CH_4 + \text{isoöctyl radical} \quad (1)$$
$$CH_3 + \text{olefin} \longrightarrow CH_3\text{.olefin radical} \quad (2)$$

It is the purpose of this communication to report our studies of reactivities of various dienes using the technique mentioned in the preceding paragraph. We were particularly interested in determining quantitatively the differences in reactivities of cumulated, conjugated, and isolated dienes. The results of our studies appear in Tables I and II.

TABLE II

Diene investigated	$E_2 - E_1$, kcal./mole	A_2/A_1
Cumulated dienes		
Allene	−2.6	0.41
Butadiene-1,2	−1.9	0.87
Conjugated dienes		
Butadiene	−3.0	25
Isoprene	−3.9	6.9

Inspection of these tables leads to the following conclusions:

A. The isolated dienes exhibit reactivities which are approximately twice as high as the reactivities of the corresponding olefins. Thus the k_2/k_1 for propylene at 65° was found to be[4] 22 while for hexadiene it is 68 (*i.e.*, 50% more than expected); the k_2/k_1 for isobutene was found to be[4] 36, while for 2,5-dimethylhexadiene-1,5 it is twice as high, 77.

B. The reactivities of cumulated dienes are surprisingly low, lower even than the reactivities of simple mono-olefins. Inspection of Table II suggests that the low frequency factor is the main reason for such a behavior. It is probable that the addition of methyl radicals to a cumulated diene takes place on the middle carbon atom. This point will be investigated further.

C. As expected the reactivities of conjugated dienes are very high, obviously due to substantial lowering of the activation energy of the addition process. It appears that the resonance energy of the allyl radical formed in the process decreases the activation energy of the addition by 2–2.5 kcal./mole as compared with simple olefins such as ethylene, propylene, etc. The reactivity of butadiene is slightly higher (even if we take into account the statical factor of 2) than that of styrene. The k_2/k_1 for styrene at 65° was found to be[3] 792 as compared with 1000 which is one half of k_2/k_1 for butadiene. The presence of methyl groups in positions 2 or 3 slightly enhances the reactivity, probably due to hyperconjugation effect which is comparable to that observed in α-methylstyrene.[3] On the other hand, substituents introduced in positions 1 and 4 exhibit a blocking effect. The case of 1,1,4,4-tetramethylbutadiene-1,3 (2,5-dimethylhexadiene-2,4) is particularly illuminating.

In conclusion we wish to thank the National Science Foundation for the support of this investigation.

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RECEIVED SEPTEMBER 19, 1957

(4) R. P. Buckley and M. Szwarc, *Proc. Roy. Soc.* (*London*) **A240**, 396 (1957).

ON THE FORMATION OF A PHOSPHORYLATED DERIVATIVE OF MEVALONIC ACID

Sir:

Since the first report by Tavormina, *et al.*,[1] that MVA[2] is an efficient precursor of cholesterol, it has been shown that (1) MVA is incorporated into squalene without prior breakdown to acetate[3–5] and that (2) ATP, Mn^{++} and a reduced pyridine nucleotide are required by a soluble yeast extract for the conversion of MVA to squalene.[6] This yeast extract has been subsequently separated into two fractions A and B, both of which are required for this conversion.[7] Evidence is now presented that fraction A catalyzes the formation of a phosphorylated derivative of MVA.

TABLE I

FORMATION OF NEW COMPOUND FROM MVA

0.5 ml. of enzyme fraction A, 116 γ of MVA (55 CPM/γ), 7 μmoles of ATP, 1 μmole of Mn^{++}, 100 μmoles of fluoride and 55 μmoles of tris-(hydroxymethyl)-aminomethane buffer of *p*H 7.4 were incubated in a total volume of 1.1 ml. 0.2 ml. aliquots were pipetted at indicated time intervals and inactivated by heating for two minutes in boiling water.

Length of incubation (minutes)	0	15	30	60
CPM in new compound	35	156	313	571
γ of MVA in new compound	0.6	2.9	5.7	10.4

TABLE II

FORMATION OF NEW COMPOUND WITH LIMITING AMOUNTS OF MVA

0.8 ml. of fraction A, approximately 100 γ of MVA (180 CPM/γ), 10μ moles of ATP, 1 μmole of Mn^{++}, 140 μmoles of fluoride and 70 μmoles of tris-(hydroxymethyl)-aminomethane buffer of *p*H 7.4 were incubated in a total volume of 1.4 ml.; 0.1 ml. aliquots were pipetted at indicated time intervals, heat inactivated and chromatographed. The slow decline in the recovery of the new compound is due to its further transformation into an as yet unidentified product.

Length of incubation (minutes)	0	30	60	120	180
CPM in new compound	38	750	723	613	591
CPM in recovered MVA	1322	661	696	700	675

1-C^{14} or 2-C^{14}-labeled MVA was incubated with ATP, Mn^{++}, fluoride and enzyme fraction A. Aliquots were withdrawn at various time intervals, heat inactivated and chromatographed on Whatman Number 2 paper with *n*-butanol–formic acid–water (77:10:13 by volume). The paper was cut into one-inch strips and eluted with water. The eluates were evaporated on a steam-bath and

(1) P. A. Tavormina, M. H. Gibbs and J. W. Huff, THIS JOURNAL, **78**, 4498 (1956).

(2) The following abbreviations are used: MVA, mevalonic acid; ATP, adenosine triphosphate.

(3) P. A. Tavormina and M. H. Gibbs, THIS JOURNAL, **78**, 6210 (1956).

(4) F. Dituri, S. Gurin and J. L. Rabinowitz, *ibid.*, **79**, 2650 (1957).

(5) J. W. Cornforth, R. H. Cornforth, G. Popjak and I. Youhotsky-Gore, *Biochem. J.*, **66**, 10 p. (1957).

(6) B. H. Amdur, H. Rilling and K. Bloch, THIS JOURNAL, **79**, 2647 (1957).

(7) H. Danielsson, B. H. Amdur and K. Bloch, unpublished results.

counted with a windowless flow counter. As shown in Table I, a new radioactive spot appeared which has a very low R_f value (0.1) and separates cleanly from MVA (R_f 0.7). Since this compound is formed from both 1-C^{14} and 2-C^{14}-labeled MVA, it must still contain the carboxyl group of MVA. When incubations were carried out with limiting amounts of 2-C^{14}-MVA, half of the MVA added was recovered even after prolonged incubation (Table II). When the remaining MVA was eluted and re-incubated with the complete yeast system[6] no squalene was formed, thus indicating that only one of the two enantiomorphs of MVA was converted into the new intermediate. When the eluted intermediate was incubated with the complete system, efficient conversion to squalene was obtained.

Examination of a chromatogram with a short wave length Mineralight revealed that the radioactive zone was free of nucleotides. The compound was thus separated from the nucleotides which remained at the origin but not from inorganic phosphate which has the same R_f value. The presence of phosphorus was established by the use of P^{32}-labeled ATP.[8] Two samples of the compound, one containing C^{14} and the other P^{32}, were isolated by chromatography and re-chromatographed with methanol–ammonia–water.[9]

(8) The P^{32}-labeled ATP was kindly prepared by Dr. Alvah H. Phillips by oxidative phosphorylation with rat liver mitochondria.

(9) R. S. Bandurski and B. Axelrod, *J. Biol. Chem.*, **193**, 405 (1951).

The C^{14}-labeled sample gave a single spot with an R_f value of 0.75. The P^{32}-labeled sample gave two spots, one corresponding to inorganic phosphate, and the other to the new compound. Comparison of its electrophoretic behavior with that of ATP and ADP[10] indicated that the compound is a monophosphorylated derivative of MVA. The chromatographic behavior of this compound was not changed by heating for 10 minutes at 100° with 1 *N* HCl or NaOH. The stability of the phosphate shows that it is not a carboxyl phosphate. The exact location of the phosphate, whether it is on C_3 or C_5, has not been ascertained.

Acknowledgments.—This work was carried out during the tenure of a scholarship from the American Cancer Society and was supported by a grant-in-aid from the National Science Foundation. The author is greatly indebted to Dr. J. M. Sprague of the Merck Sharp and Dohme Laboratories for a generous gift of samples of mevalonic acid to this laboratory. The author also wishes to thank Dr. K. Bloch and co-workers for making available their unpublished results as well as for many helpful discussions.

(10) H. Hilz and F. Lipmann, *Proc. Natl. Acad. Sci.*, **41**, 880 (1955).

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RECEIVED NOVEMBER 1, 1957

BOOK REVIEWS

Light Vegetation and Chlorophyll. By J. TERRIEN, G. TRUFFAUT and J. CARLES. Translated by MADGE E. THOMPSON. Philosophical Library, Inc., 15 East 40th Street, New York 16, N. Y. 1957. 228 pp. 12.5 × 19 cm. Price, $6.00.

This book is divided into two sections, the first based on "Lumière et Végétation" by Terrien and Truffaut, the second on "L'Énergie Chlorophyllienne" by Carles. The first section contains a thorough description of solar radiation, natural light fields and light absorption by leaves, and following this, eight chapters concerning the various effects of light on plants, including photosynthesis, phototropism and photoperiodism. The second section, by Carles, is an essay on photosynthesis which is in part a duplication of some of the material in the first section.

The authors "have tried to give the reader an idea of what is known of the relationship between light and vegetation," and in this they have succeeded fairly well. The introductory chapters are complete and contain quite a bit of useful reference material, and the chapter entitled "Photosynthesis and Photography" is a good, elementary description of electron conduction in crystals, a subject which is currently of great interest in the field of photosynthesis.

On the other hand, since the range of topics covered is very broad, the treatment is necessarily too sketchy in some places, particularly in the chapters on photoperiodism and phototropism. Furthermore, the style is diffuse, the translation is rough in places and there are a number of errors and omissions.

The major criticism of this book, however, is that it is badly out of date. This is largely due to the fact that the two original works on which it is based are six and four years old, respectively, and evidently were not rewritten before being combined in this edition. Chiefly for this reason, the book will be of limited value to the research worker in the field, or to the chemist who is interested in a short authoritative monograph.

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Amino Acid Handbook. Methods and Results of Protein Analysis. By RICHARD J. BLOCK, Ph.D., Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York, and Department of Biochemistry, New York Medical College, New York, with the cooperation of Kathryn W. Weiss, A.B., The Borden Company, Yonkers, New York. Charles C Thomas, 301–327 East Lawrence Avenue, Springfield, Illinois, 1956. xiii + 386 pp. 16 × 23.5 cm. Price, $10.50.

1. This monograph has a twofold objective of describing "tried and proven examples of the three most widely used methods of amino acid analysis, *i.e.*, by microörganisms, by column chromatography and by paper chromatography," in sufficient detail "without the need of recourse to the original literature," and to tabulate "the amino acid composition of proteins, biologically active polypeptides and foods."

2. The first objective is set forth in 167 pages, followed by a short chapter on Protein and Amino Acid Consumption in the United States and concluded with a 66 page bibliography, listing approximately 1200 or more references. The reviewer feels that the first objective, though laudable is ambitious almost beyond attainment, for it is probably true that the clearest exposition of a method is usually to be found