(OH^- form). Following a water wash the column was eluted with 1 *N* acetic acid to give 383 mg. of brown solid which yielded a single Koenig positive spot (R_f 0.15) upon paper chromatography with 0.5 *N* ammonia water (1 vol.)–95% ethanol (1 vol.)–*n*-butanol (4 vol.). The spot corresponds to that obtained with authentic γ-(3-pyridyl)-γ-methylaminobutyric acid.[5] The brown solid was heated to 155° under nitrogen to give 43 mg. of clear chloroform-soluble oil, $\lambda_{max.}$ 262 mμ. The optical density of the oil in methanol corresponded to that calculated for 48 mg. of cotinine (5-(3′-pyridyl)-1-methylpyrrolidone-2). Paper chromatography in the aforementioned system and also in *sec*-butyl alcohol (45 vol.)–formic acid (8.4 vol.)–water (6.6 vol.) gave major Koenig positive spots corresponding to those of authentic cotinine. The oil yielded a yellow picrate with m.p. 104–106° corresponding to that of authentic continine picrate. The mixed melting point showed no depression.

Anal. Calcd. for $C_{16}H_{15}N_5O_8$: C, 47.41; H, 3.73; N, 17.28. Found: C, 47.49; H, 3.80; N, 17.19. The infrared spectra of authentic and isolated picrates in Nujol mulls were identical.[6] γ-(3-Pyridyl)-γ-methylaminobutyric acid (based on the amount of the lactam cotinine) accounts for approximately 5% of the administered nicotine.

It has been observed[7,8] that the urine of dogs following the administration of nicotine contains a substance insoluble in ether, which gives directly a red color with cyanogen bromide. Since γ-(3-pyridyl)-γ-methylaminobutyric acid gives this color reaction[4] and is insoluble in ether, the isolation of this acid from urine explains, in part at least, the appearance of the color. Control dog urine yields neither the color reaction nor the methylamino acid.

Thermal cyclization of γ-(3-pyridyl)-γ-methylaminobutyric acid from urine resulted in the formation of cotinine with $[\alpha]^{30}_{5461}$ −18.77° in methanol. A sample of γ-(3-pyridyl)-γ-methylaminobutyric acid prepared *in vitro* from (−)-nicotine[5] was cyclized under similar conditions to give cotinine with $[\alpha]^{21.5}_{5461}$ −18.16°. These two rotations are of the same sign and order of magnitude as that of cotinine prepared from (−)-nicotine by the method of Pinner.[5] It is inferred, therefore, that in the metabolic processes leading to the formation of γ-(3-pyridyl)-γ-methylaminobutyric acid the optical configuration of the asymmetric carbon atom of (−)-nicotine is retained.

The urine of dogs receiving (−)-nicotine contains cotinine in addition to γ-(3-pyridyl)-γ-methylaminobutyric acid and other metabolites, as demonstrated by paper chromatography and chloroform extraction. Studies *in vitro* with γ-(3-pyridyl)-γ-methylaminobutyric acid showed that aqueous solutions at *p*H 7 and below spontaneously yield cotinine at room temperature. In fresh samples of urine, voided usually in the region of *p*H 6, the γ-(3-pyridyl)-γ-methylaminobutyric acid fraction was greater than in older samples in which the cotinine fraction had become larger. Consequently, cotinine in the urine of dogs may be entirely an artifact which arose from the spontaneous lactamization. An attractive alternate explanation for the appearance of the lactam is the possibility that, in the metabolism of nicotine, cotinine is an intermediate which can in subsequent enzymatic reactions be hydrolyzed to γ-(3-pyridyl)-γ-methylaminobutyric acid.

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(6) Kindly obtained by Mr. W. B. Wartman, Jr., The American Tobacco Company Research Laboratory.

(7) P. S. Larson and H. B. Haag, *J. Pharmacol. Exp. Therap.*, **76**, 240 (1942).

(8) P. S. Larson, H. B. Haag and J. K. Finnegan, *ibid.*, **86**, 239 (1946).

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METHYL AFFINITIES OF DIENES

Sir:

A comparatively simple technique, developed in our laboratories some years ago and described in previous communications,[1-3] permits us to measure

TABLE I

Compound	T, °C.	k_2/k_1	No. of exp.	Range of mole % of the investigated comp.
Cumulated dienes				
Allene	54.8	20.3 ± 0.2	3	3.4–6.3
Allene	64.9	17.6 ± 0.2	3	3.4–6.3
Allene	75.0	16.0 ± 0.2	3	3.4–6.3
Allene	85.1	14.3 ± 0.2	3	3.4–6.3
Butadiene-1,2	54.8	17.2 ± 1.0	4	2.2–8.8
Butadiene-1,2	64.9	14.8 ± 2.0	5	2.9–7.4
Butadiene-1,2	75.0	13.4 ± 1.0	5	2.0–9.2
Butadiene-1,2	85.1	13.5 ± 1.0	6	2.2–6.5
Conjugated Dienes				
Butadiene-1,3	54.8	2350 ± 35	4	0.06–0.15
Butadiene-1,3	64.9	2015 ± 30	3	.06– .15
Butadiene-1,3	75.0	1790 ± 40	3	.07– .12
Butadiene-1,3	85.1	1630 ± 10	3	.07– .14
Isoprene	54.8	2460 ± 70	3	0.08–0.16
Isoprene	64.9	2090 ± 50	4	.08– .16
Isoprene	75.0	1800 ± 30	4	.04– .16
Isoprene	85.1	1470 ± 30	3	.04– .16
2,3-Dimethylbutadiene-1,3	64.9	2230 ± 70	4	0.07–0.21
1,4-Diphenylbutadiene-1,3	64.9	378 ± 6	3	0.06–0.13
2,5-Dimethylhexadiene-2,4	64.9	21.3[a]	7	0.2–7.0
1,1,4,4-Tetraphenyl butadiene-1,3	64.9	~60[b]		
Isolated Dienes				
Hexadiene-1,5	64.9	68[a]	9	1.0–7.7
2,5-Dimethylhexadiene-1,5	64.9	77[a]	6	1.0–6.5

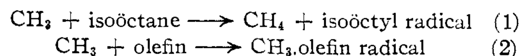
[a] k_2/k_1 determined by the extrapolation to zero monomer concentration, using the procedure described by Buckley, Leavitt and Szwarc, THIS JOURNAL, **78**, 5557 (1956). [b] This compound was investigated in toluene since it is insoluble in isoöctane. The results were recalculated for isoöctane solution.

(1) M. Levy and M. Szwarc, THIS JOURNAL, **77**, 1949 (1955).

(2) M. Szwarc, *J. Polymer Sci.*, **16**, 367 (1955).

(3) F. Leavitt, M. Levy, M. Szwarc and V. Stannett, THIS JOURNAL, **77**, 5493 (1955).

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.

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

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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 pH 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 pH 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. Youbotsky-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.