methyl cytosine (HMC) nucleoside, in the face of good yields of the deoxyribonucleosides of the other three bases, was reported by Cohen,[2] while the seemingly unrelated work of Jesaitis and Goebel[3] indicated the presence of a significant quantity of glucose in T4 DNA. With the notion that the presence of glucose in the DNA molecule somehow inhibited the action of the combined enzymes, we have attempted to separate the products of such enzymatic hydrolysates by ion-exchange and to identify them. Such experiments have led us to the conclusion that glucose is associated mole for mole with HMC in T4 DNA and, further, that the glucose is probably linked as an O-glycoside to the 5-hydroxymethyl group of HMC, rather than substituted for deoxyribose in the DNA sugar-phosphate chain. We have extended the observation of Jesaitis and Goebel of the presence of glucose in T4 to T2 and T6 DNA's (but not T1 DNA) and have noted that the ratio of glucose to DNA phosphorus is about 0.17 in these phages.

The course of hydrolysis of 40 mg. of highly purified T4[4] DNA by pancreatic DNase was followed by electrometric titration. A maximum of 11% of the phosphoryl linkages was hydrolyzed by the enzyme, this value being about half of that obtained by a similar digestion of DNA from other sources.[5] Half of the digest was hydrolyzed with whole *Crotalus adamanteous* venom (containing both diesterase and 5′-monoesterase) and the other half was hydrolyzed with a diesterase preparation essentially free of monoesterase activity.[6] Both hydrolyses were again followed by titration and reached an end-point when an additional 70% of the phosphoryl linkages of the DNase-produced polynucleotides was broken.

With calf thymus DNA such procedures yield nucleosides and 5′-nucleotides, respectively, in quantitative yield.[6] With T4 DNA, however, many polynucleotides remain.

The ion-exchange analyses[7] of the two digests are summarized in Table I. It is clear that the diesterase does not cleave most of the HMC nucleotide linkages. However, a variety of nucleosides, nucleotides and polynucleotides containing HMC was obtained. Within each such compound or mixture of compounds, HMC was determined by spectrophotometric observations and glucose by the anthrone test.[8] Wherever HMC was present, glucose was also present in equimolar quantity. In addition, there was at least one HMC residue per polynucleotide.

TABLE I

PER CENT RECOVERY OF THE BASES OF T4 DNA BY ION-EXCHANGE ANALYSIS FOLLOWING THE COMBINED ACTION OF PANCREATIC DNASE AND (a) WHOLE SNAKE VENOM AND (b) SNAKE VENOM DIESTERASE

Base	Nucleoside	Mononucleotide	Polynucleotide
	(a) Whole venom — Per cent recovered as		
(HMC)[b]	4	12	84
Guanine	79	0	21
Thymine	72	0	27
Adenine	78	0	21
	(b) Venom diesterase		
(HMC)[b]	..	17	82
Guanine	8	74	18
Thymine	9	64	26
Adenine	8	72	19

[a] Nucleoside formation with diesterase is ascribed to residual 5′-monoesterase action. [b] 5-hydroxymethylcytosine.

(4) R. M. Herriott and J. L. Barlow, *J. Gen. Physiol.*, **36**, 17 (1952).
(5) M. Kunitz, *ibid.*, **33**, 363 (1949–1950).
(6) R. O. Hurst and G. C. Butler, *ibid.*, **193**, 91 (1951).
(7) E. Volkin and W. E. Cohn, *ibid.*, **205**, 767 (1953).

Glucose was identified as such after liberation from T4 DNA by boiling for 7 hours with a sulfonic acid cation exchanger (Dowex-50-H). The identification was made by anion-exchange chromatography in a borate system,[9] and by the hexokinase-firefly luminescence system.[10] These analyses agree with each other and with the colorimetric anthrone method[8] in indicating about 90% release of the glucose as such.

Indirect evidence on the mode of linkage of glucose to HMC in T4 was given by conventional acid hydrolysis (1 *N* HCl, 1 hour, 100°), which yielded 88% of the glucose as free sugar whereas 87% of the HMC remained in mononucleotide, nucleoside diphosphate and polynucleotide form. From the latter observation, glucose cannot be the sugar involved in internucleotidic linkage of HMC. Furthermore, its appearance without simultaneous HMC destruction is more consistent with the properties of O-glycosides than with those of pyrimidine-N-glycosides, which are markedly acid-stable. The sugar is therefore tentatively allocated to the 5-hydroxymethyl group.

NOTE ADDED IN PROOF.—Recently a report of similar findings with T2 bacteriophage DNA has appeared by Sinsheimer, *Science*, **120**, 551 (1954), dealing with the identification of glucose associated with BMC mononucleotide. Our results confirm this and extend the observation to the polynucleotide-bound BMC.

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(9) J. X. Khym and L. P. Zill, THIS JOURNAL, **74**, 2090 (1952).
(10) B. L. Strehler and J. R. Totter, *Arch. Biochem. and Biophys.*, **40**, 28 (1952).

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RECEIVED OCTOBER 4, 1954

C^{14} AND C^{13} INTRAMOLECULAR ISOTOPE EFFECTS IN THE DECARBOXYLATION OF LIQUID MALONIC ACID AT 140.5°

Sir:

The theoretical value for the ratio of C^{14} to C^{13} isotope effects in reactions involving rupture of C-C, C-N or C-O bonds is near 2.[1] Reported experimental values are: decarboxylation of mesitoic acid[2] -2.8 ± 0.3; dehydration of oxalic acid[3] -2.0 ± 0.2; enzymatic hydrolysis of urea[4] -3.2 ± 0.4; non-enzymatic hydrolysis of urea[5] -1.8 ± 0.2; decarboxylation of malonic acid[6] -3.5 ± 0.4. Only for malonic acid are other results available for the C^{14} isotope effect; Roe and Hellman[7] gave a figure of $6 \pm 2\%$ for the intramolecular

(1) J. Bigeleisen, *J. Phys. Chem.*, **56**, 823 (1952).
(2) W. H. Stevens, J. M. Pepper and M. Lounsbury, *J. Chem. Phys.*, **20**, 192 (1952).
(3) A. Fry and M. Calvin, *ibid.*, **56**, 901 (1952).
(4) J. A. Schmitt, A. L. Myerson and F. Daniels, *ibid.*, **56**, 917 (1952).
(5) J. A. Schmitt and F. Daniels, THIS JOURNAL, **75**, 3564 (1953).
(6) P. E. Yankwich and E. C. Stivers, *J. Chem. Phys.*, **21**, 61 (1953).
(7) A. Roe and M. Hellman, *ibid.*, **19**, 660 (1951).

effect, a value which stands in approximate theoretical agreement with the 2.92 ± 0.07% result for the related C^{13} effect according to a recent study.[8] Since the isotope effect ratio 2 is a clear prediction of the absolute rate theory, we have attempted to establish more firmly whether the malonic acid case does deviate by redetermining the C^{13} and C^{14} intramolecular isotope effects, employing only mass spectrometric measurements. The results given below are preliminary.

Labeled malonic acid was synthesized *via* the cyanide-cyanoacetate route employed previously.[6] The crude malonic acid was purified on a silicic acid column, using the *n*-butanol-chloroform solvent system[9]; after preliminary concentration, the product was freed of solvents by sublimation *in vacuo* at 95–100°. This material was diluted to the desired isotopic composition (about 1% C^{14}, total carbon basis) with Eastman Kodak Co. White Label acid which had been subjected to the same manipulations. The mixture was recrystallized from acetone–benzene, resublimed, then stored at room temperature in a vacuum desiccator. The batch decomposition, combustion and degradation techniques employed have been described in detail elsewhere[8]; all samples of carbon dioxide submitted for mass analysis were equilibrated first with standard water to eliminate the effect of variation in O^{18} content.

TABLE I

MOLE FRACTIONS FROM ION CURRENT RATIOS × 10^6

	CO_2 evolved	Acetic acid combustion	Methyl carbon of acetic acid
	10420	10684	10661
C^{13}	10416	10675	10657
	10424	10681	
	16313	8543	
C^{14}	16290	8581	
	16299	8645	

In Table I are given the C^{13} and C^{14} mole fractions obtained from three runs in which yields were nearly quantitative; the results for carboxyl carbon of acetic acid were erratic, however. From the data shown we calculate for the C^{14} effect 5.45 ± 0.46%, for the C^{13} effect 2.85 ± 0.09%; the ratio of these is 1.91 ± 0.17. Since the two heavy isotopes are present in approximately the same concentration in the carboxyl carbon atoms, the results obtained for C^{13} depend upon those for C^{14}; accordingly, agreement of the present C^{13} figure with that obtained previously by the same techniques applied to unlabeled starting material[8] increases our confidence in the C^{14} result. We believe that these results indicate strongly that the malonic acid case can no longer be considered deviant from the predictions of the absolute rate theory.

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RECEIVED SEPTEMBER 10, 1954

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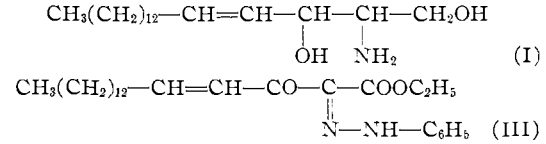
(9) C. S. Marvel and R. D. Rands, *ibid.*, **72**, 2642 (1950).

THE SYNTHESIS OF SPHINGOSINE

Sir:

The structure of sphingosine (I) was conclusively established by Carter and his co-workers.[1] Evidence for the *trans* configuration of the double bond has been presented recently by other investigators.[2,3] We wish to announce the synthesis of *trans*-DL-*erythro*-1,3-dihydroxy-2-amino-4-octadecene (I).

Ethyl α-(*trans*-2-hexadecenoyl)-acetoacetate (II) (m.p. 33–34°) was obtained in a 75% yield from the acid chloride and ethyl acetoacetate. *Anal.* Calcd. for $C_{22}H_{38}O_4$: C, 72.1; H, 10.4. Found: C, 72.2; H, 10.6. The Japp–Klingemann reaction[4] of (II) with benzenediazonium chloride afforded a 60–70% yield of the α-phenylazo ester (III), m.p. 39–41°. *Anal.* Calcd. for $C_{26}H_{40}N_2O_3$: C, 72.8; H, 9.3; N, 6.5. Found: C, 72.9; H, 9.5; N, 6.2.



(I)

(III)

Reductive acetylation with zinc in acetic acid gave a quantitative yield of the corresponding α-acetamido ester, m.p. 62–63°. *Anal.* Calcd. for $C_{22}H_{39}NO_4$: C, 69.3; H, 10.2; N, 3.6. Found: C, 69.8; H, 10.6; N, 4.0.

Selective reduction of the β-oxo group was effected with sodium borohydride under mild conditions. The two diastereoisomeric carbinols could be separated by crystallization. One of them (m.p. 64–65°) was obtained in a pure state. *Anal.* Calcd. for $C_{22}H_{41}NO_4$: C, 68.9; H, 10.7; N, 3.6. Found: C, 68.9; H, 10.8; N, 3.6.

Saponification of the pure isomer with diluted hydrochloric acid yielded ethyl 2-amino-3-hydroxy-4-octadecenoate hydrochloride, m.p. 110–112°. *Anal.* Calcd. for $C_{20}H_{40}NO_3Cl$: C, 63.4; H, 10.7; N, 3.7; Cl, 9.4. Found: C, 63.5; H, 10.8; N, 4.0; Cl, 9.6.

Direct treatment of the hydrochloride with an excess of lithium aluminum hydride gave the desired 1,3-dihydroxy-2-amino-4-octadecene (I). The *erythro* configuration of this base was proven by its hydrogenation to the corresponding dihydrosphingosine.

The triacetyl derivative of (I) melted sharply at 90–91° after one crystallization from methanol. *Anal.* Calcd. for $C_{24}H_{43}NO_5$: C, 67.7; H, 10.1; N, 3.3. Found: C, 67.9; H, 10.3; N, 3.6.

The presence of an allylic system involving the carbon atoms 3, 4 and 5 was demonstrated by hydrogenolysis of the secondary acetoxy group (1). When the triacetyl derivative was shaken with platinum oxide and hydrogen, approximately 50–60% of the theoretical amount of acetic acid was produced.

The infrared spectrum of the triacetyl compound showed the characteristic *trans*-peak[2] near 10.3μ.

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(2) K. Mislow, THIS JOURNAL, **74**, 5155 (1952).

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(4) R. Japp and F. Klingemann, *Ber.*, **20**, 2942, 3284, 3398 (1887).