polyacetylenes. Except in the case of Id, only small amounts of the linear coupling products result.

For example 1 part of nona-1,8-diyne (Ia) in 100 parts of pyridine was heated and stirred with 15 parts of neutral cupric acetate at 55° for 3 hours. The product on chromatography on 200 parts of alumina into *ca.* 300 fractions gave successively: (a) the C_{18}-tetrayne IIa (10%), m.p. 210–212° (identical with that made previously[1,2b]); (b) the C_{27}-hexayne IIIa (13%), m.p. 125–126° (hydrogenated to $C_{27}H_{54}$,[4] m.p. 47–48°, mol. wt., 374[5]; calcd. 378); (c) the C_{36}-octayne IVa (11%), m.p. 135–136° (hydrogenated to $C_{36}H_{72}$,[4] m.p. 70–71°, mol. wt., 517; calcd., 504); (d) the C_{45}-decayne Va (4%), m.p. 144–145° (hydrogenated to $C_{45}H_{90}$,[4] m.p. 78.5–79°, mol. wt., 618; calcd., 630); (e) probably the C_{54}-dodecayne VIa (4%), m.p. 144–145° (hydrogenated to $C_{54}H_{108}$,[4] m.p. 90–91°, mol. wt. not determined due to insolubility).

A similar coupling of octa-1,7-diyne (Ib) gave: (a) the C_{16}-tetrayne IIb (9%), m.p. 162–163° (identical with that made previously[2a]); (b) the C_{24}-hexayne IIIb (14%), m.p. 173–174° (hydrogenated to $C_{24}H_{48}$, m.p. 46.5–47°, mol. wt., 330; reported: m.p. 46–47°, mol. wt., 336); (c) the C_{32}-octayne IVb (8%), m.p. 154–155° (hydrogenated to $C_{32}H_{64}$, m.p. 58–59°, mol. wt., 429; reported: m.p. 59–60°, mol. wt., 448); (d) the C_{40}-decayne Vb (9%), m.p. 155–157° (hydrogenated to $C_{40}H_{80}$,[4] m.p., 74–75°, mol. wt., 568; calcd. 560). Coupling of hepta-1,6-diyne (Ic) did not give the C_{14}-tetrayne IIc (if formed, it probably decomposed),[1,2b] but the C_{21}-hexayne IIIc (3%), m.p. 174–175° (hydrogenated to $C_{21}H_{42}$,[4] m.p. 63–64°, mol. wt., 289; calcd. 294) and the C_{28}-octayne IVc (4%), m.p. 213–214° (hydrogenated to $C_{28}H_{56}$, m.p. 47–48°, mol. wt., 389; reported: m.p. 47–48°, mol. wt., 398).

Coupling of hexa-1,5-diyne (Id) and chromatography into 450 fractions gave: (a) the linear dimer (9%), m.p. 98–99° (identical with that made previously[1,2b]); (b) the C_{18}-hexayne IIId (6%), decomposes on heating (hydrogenated to $C_{18}H_{36}$, m.p. 72–73°, identical to that obtained from IIa); (c) the C_{24}-octayne IVd (6%), decomposes on heating (hydrogenated to $C_{24}H_{48}$, m.p. 46–47°, identical to that obtained from IIIb); (d) the C_{30}-decayne Vd (6%), decomposes on heating (hydrogenated to $C_{30}H_{60}$, m.p. 57–58°; mol. wt., 416; reported m.p. 57–58°, mol. wt., 420); (e) probably the cyclic C_{42}-tetradecayne, decomposes on heating (hydrogenated to $C_{42}H_{84}$,[4] m.p. 75–76°, mol. wt., 598; calcd., 588).

All the above cyclic polyacetylenes were highly crystalline. They were shown to differ from each other since they gave mutual depressions in m.p. Their cyclic nature was shown by the absence of acetylenic hydrogen (no band at *ca.* 3300 cm.$^{-1}$ in the infrared, no precipitate with silver nitrate) and by the absence of terminal methyl groups (no band at *ca.* 1380 cm.$^{-1}$) in the corresponding saturated compounds. The latter gave satisfactory analytical results, but most of the polyacetylenes exploded on attempted combustion and gave low carbon values. In all the four series studied, cyclic polyacetylenes of higher mol. wt. than those described were also obtained; their structures are now under investigation.

The present method makes available in one step highly unsaturated large ring alicyclic hydrocarbons (and by hydrogenation the corresponding saturated ones) over a wide range of size, including considerably larger ones than the thirty-four membered cycle which is the biggest alicyclic ring prepared previously.

DANIEL SIEFF RESEARCH INSTITUTE
WEIZMANN INSTITUTE OF SCIENCE
REHOVOTH, ISRAEL
FRANZ SONDHEIMER
YAACOV AMIEL
REUVEN WOLOVSKY

RECEIVED JUNE 17, 1957

(4) This is a previously unknown cycloalkane.

(5) All molecular weights were determined by the Rast method in camphene.

THE CHANGE OF OPTICAL ACTIVITY OF POLY-γ-BENZYL-L-GLUTAMATE IN AN ELECTRIC FIELD

Sir:

The optical activity of a molecule is different along different directions. Therefore we expect the optical activity of a solution of these molecules to change if the molecules are oriented. However, this effect has not been described. We wish to report the change of optical activity of a solution of poly-γ-benzyl-L-glutamate (PBLG) caused by orientation of the molecules in an electric field.

An equation relating the change in optical activity to molecular parameters at infinite dilution has been derived.[1] For a helical molecule the

$$\frac{[\alpha]_E - [\alpha]_0}{E^2} = \frac{2}{45}([\alpha_{33}) - [\alpha_{11}])[p_3^2 + (q_{33} - q_{11})]$$

change in the specific optical activity in an electric field[2] E is proportional to the difference in optical activity for light incident parallel to the helical axis $[\alpha_{33}]$ and perpendicular to the helical axis $[\alpha_{11}]$. The proportionality factor is the electrical orientation term which involves the permanent and induced dipole moments of the molecule. The specific optical activity in the absence of a field $[\alpha]_0$ is just $(2[\alpha_{11}] + [\alpha_{33}])/3$.

In order to measure the change of optical activity in an electric field, the light must be incident parallel to the field. Only along this direction is the refractive index and optical absorption the same for all directions of the plane of polarization.[3]

A one-cm. cell with transparent conducting glass[5] electrodes was used. The optical rotation with the electric field off, then on, was measured from λ = 330 to 550 mμ in a Rudolph automatic spectropolarimeter[6] for two concentrations of M_w = 64,000 PBLG[7] in ethylene dichloride. The

(1) I. Tinoco, Jr., and W. G. Hammerle, *J. Phys. Chem.*, **60**, 1619 (1956).

(2) The units of E are e.s.u./cm. (1 e.s.u. = 300 volts).

(3) The change of refractive index in an electric field is electrical birefringence or Kerr effect.[4] The change of optical absorption is electrical dichroism; it has not been reported.

(4) C. G. LeFèvre and R. J. W. LeFèvre, *Revs. Pure Appl. Chem.*, **5**, 261 (1955).

(5) We wish to thank Dr. E. M. Greist of Corning Glass Works for kindly furnishing this glass (E-C #7740).

(6) We wish to thank Professors J. B. Nielands and H. K. Schachman, and Mr. B. Burnham for the use of this instrument.

(7) We wish to thank Dr. E. R. Blout for kindly supplying a sample of PBLG (#ES-508) for these measurements.

rotatory dispersion in the absence of the field was in good agreement with the measurements of Doty and Yang.[8,9] In the presence of the electric field an increase in optical rotation was observed at all wave lengths. The change was found to be proportional to the square of the electric field strength as predicted; an easily measurable change of about 0.1° occurred for a field of about 2,000 volts/cm.

As the electrical orientation term is not known, quantitative values of $[\alpha_{33}]$ and $[\alpha_{11}]$ cannot be obtained. However, an estimate of this term from electrical birefringence studies[10] allows us to make the following conclusions. Both $[\alpha_{33}]$ and $[\alpha_{11}]$ are very large in absolute magnitude, but $[\alpha_{33}]$ is positive and $[\alpha_{11}]$ is negative. Both values increase in absolute magnitude as the wave length decreases; however $[\alpha_{11}]$ increases faster, thus leading to the change in sign of the average optical activity $[\alpha]_0$ at $\lambda = 425$ mμ. These conclusions are in good agreement with Moffitt's prediction.[11]

(8) P. Doty and J. T. Yang, THIS JOURNAL, **78**, 498 (1956).
(9) J. T. Yang and P. Doty, *ibid.*, **79**, 761 (1957).
(10) I. Tinoco, Jr., *ibid.*, in press.
(11) W. E. Moffitt, *Proc. Natl. Acad. Sci., U. S.*, **42**, 736 (1956).

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF CALIFORNIA IGNACIO TINOCO, JR.
BERKELEY, CALIFORNIA

RECEIVED MAY 15, 1957

VITAMIN B_{12} AND PROTEIN BIOSYNTHESIS. II. EFFECT OF VITAMIN B_{12} ON AMINO ACID INCORPORATION IN MICROSOMAL PREPARATIONS

Sir:

It has been reported from this laboratory that, while vitamin B_{12} has no effect on nucleic acid biosynthesis,[1] there is a decreased incorporation of C^{14}-labeled amino acids into liver protein in vitamin B_{12}-deficient animals,[2,3] indicating that vitamin B_{12} is involved in the incorporation of amino acids into protein.

Following these findings with the whole animal, the incorporation of C^{14}-labeled amino acids into protein has been studied *in vitro* using the microsomal fraction of liver and of spleen from both B_{12}-deficient and normal rats.

The rats were killed by decapitation and the livers removed and microsomes prepared by the procedure of Zamecnik and Keller.[4] This microsome fraction was freed only of mitochondria and nuclei since, as Keller and Zamecnik[5] have reported, the supernatant fraction is essential along with microsomes for the incorporation of amino acids into protein. These preparations containing microsomes and supernatant were then incubated under oxygen (95% O_2, 5% CO_2) with fructose diphosphate, C^{14}-amino acid, ATP[6] and GTP[6] for one hour in the Dubnoff shaker. They were then inactivated by the addition of TCA,[6] and the proteins were isolated and counted at infinite thickness in a GM gas-flow counter. The results are given in Table I.

(1) S. R. Wagle and B. C. Johnson, *Federation Proc.*, **16**, 401 (1957).
(2) S. R. Wagle and B. C. Johnson, *Arch. Biochem. Biophys.*, in press.
(3) Presented in part before annual meeting, National Vitamin Foundation, March 6, 1957, New York; B. C. Johnson, *Am. J. Clin. Nutrition*, in press.
(4) P. C. Zamecnik and E. B. Keller, *J. Biol. Chem.*, **209**, 337 (1954).
(5) E. B. Keller and P. C. Zamecnik, *ibid.*, **221**, 45 (1956).

TABLE I

INCORPORATION OF C^{14}-AMINO ACIDS INTO PROTEIN BY MICROSOME PREPARATIONS[a] FROM THE LIVER AND THE SPLEEN OF VITAMIN B_{12}-DEFICIENT AND NORMAL RATS[b]

	Liver microsome preparation, c.p.m./mg. protein		Spleen microsome preparation, c.p.m./mg. protein	
B_{12} status	$-B_{12}$	$+B_{12}$	$-B_{12}$	$+B_{12}$
$C^{14}H_3$-Methionine	16	76	26	94
2-C^{14}-Alanine	12	57	21	69

[a] Complete system contained 0.1 μM. FDP (6), 0.5 μM. ATP, 0.25 μM. GTP, 0.5 ml. of microsome preparation and labeled amino acid, made to 1 ml. with 0.15 molar KCl. [b] Each figure is the mean for three rats and run in duplicate. Excellent agreement was obtained between replicate animals.

In another series of experiments, the enzyme preparations were supplemented with vitamin B_{12}. The results are given in Table II.

TABLE II

THE EFFECT OF ADDITION OF VITAMIN B_{12} TO LIVER AND SPLEEN MICROSOME PREPARATIONS[a] ON THE INCORPORATION OF C^{14}-AMINO ACIDS INTO PROTEIN[b]

B_{12} status of animals	B_{12} added to microsome prep.	Liver microsome preparation, c.p.m./mg. protein		Spleen microsome preparation, c.p.m./mg. protein	
		$-B_{12}$	$+B_{12}$	$-B_{12}$	$+B_{12}$
C^{14}-Methionine	None	19	64	14	81
C^{14}-Methionine	50 mμg	53	73	51	88
2-C^{14}-Alanine	None	12	44	21	67
2-C^{14}-Alanine	50 mμg	40	66	48	90

[a] See Table I. [b] Each figure is the mean for two rats, run in duplicate. Agreement was excellent between animals.

Table I clearly shows that there is much less incorporation of labeled amino acids into protein in the case of microsome preparations prepared from the livers and spleens of vitamin B_{12}-deficient rats than in those from normal animals. These results agree with our previous data on intact animals.[2] In an additional experiment, in which the effect of level of substrate (labeled amino acid) concentration on incorporation was studied, it was found that an increase in the incorporation of amino acids was obtained with microsome preparations (both liver and spleen) from B_{12}-normal animals, upon incubation with increasing levels of amino acid, but that these substrate increments had no effect in preparations from deficient animals. This indicates an enzymatic block in protein synthesis in deficient animals. Again, when the microsome preparations, particularly from the deficient animals, were supplemented with crystalline vitamin B_{12} increased incorporation of the amino acids occurred, bringing the incorporation almost to a normal level (the stepwise increase obtained with graded levels of vitamin B_{12} and also amino acid increment data will be reported in full

(6) ATP = adenosine triphosphate; GTP = guanosine triphosphate; TCA = trichloroacetic acid; FDP = fructose diphosphate.