idence which demonstrates that the formation of 2-ketopantoate from 2-ketoisovalerate proceeds stereospecifically with *inversion* of the configuration at C-3 of 2-ketoisovalerate.

Freshly grown cells of a valine-isoleucine auxotroph of *E. coli* (ATCC 23783) were incubated with [4-^{13}C]-(2*RS*,3*S*)valine[4] (**4**, 20 mg, 0.17 mmol, 90 atom % ^{13}C) in a nitrogen-free medium containing β-alanine.[5-9] After the termination of the incubation, calcium pantothenate (20 mg, 0.042 mmol) was added, and, following hydrolysis (dilute H_2SO_4), pantolactone **5** (9 mg) was isolated. The ^{13}C NMR (benzene-d_6 solution) of the biosynthesized pantolactone **5** showed a ca. fourfold enhancement of the *downfield* methyl signal (δ 22.49). In contrast, the intensities of the upfield methyl signal (δ 18.78) and of other signals were unchanged. It is reasonable to assign the downfield methyl signal of pantolactone at δ 22.49 at the methyl group cis to the C-2 hydroxyl group.[10] Since C-2 of pantolactone has the *R* configuration,[11] it follows that the biosynthetic product was labeled in the *re*[12] methyl group, as shown in **5**. Hence the configuration at C-3 of chirally labeled 2-ketoisovalerate, derived in vivo from chiral valine **4**, was inverted in the course of ketopantoate formation.

It is of some interest to note that no randomization of the ^{13}C labeling was observed in this process. This result contrasts with our earlier studies on the stereochemistry of the catabolism of chirally labeled valines in rat liver preparations. In rat livers, complete randomization of the labeling occurred in the course of conversion of chiral valines to isobutyrate,[13] presumably via enolization of 2-ketoisovalerate.[14]

It is also of interest to note that the observed stereochemistry of 2-ketopantoate biosynthesis contrasts with the stereochemistry of the serine hydroxymethyltransferase reaction, in which glycine and N^5,N^{10}-methylenetetrahydrofolate react to give L-serine and tetrahydrofolate with *retention* of configuration at the α carbon of the glycine unit.[15]

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(6) The nature of the metabolic block in this auxotroph is unknown. However, as it has a requirement for valine and isoleucine, *but not leucine*, it clearly is not deficient in the aminotransferase interconverting the branched-chain keto acids and amino acids, since apparently the same aminotransferase interconverts the three pairs of keto acids and amino acids.[7] It follows that strain ATCC 23783 must be defective at some stage leading to the formation of 2-ketoisovalerate (and 2-keto-3-methylvaleric acid). Consequently the only source of 2-ketoisovalerate for pantoate biosynthesis is the exogenous valine. Therefore, the biosynthesized pantoate should have the same isotopic enrichment as the exogenous valine, as was observed by Teller[5] using a different *E. coli* valine-isoleucine auxotroph.
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(8) In a preliminary experiment, cells of *E. coli* (ATCC 23783) were obtained from two 100-mL cultures freshly grown to maximum O.D. over 24 h at 37 °C, 250 rpm, in medium I.[9] The cells were collected by centrifugation, resuspended in 200 mL of medium II, recentrifuged, and then resuspended in 100 mL of medium II. To this was added [4-^{14}C]-DL-valine (20 mg, 6 × 10^6 cpm), and the suspension was incubated at 37 °C, 250 rpm, for 24 h. Then calcium pantothenate (20 mg) was added and the mixture was acidified, pH 1.3, with concentrated H_2SO_4. Additional H_2SO_4 (6 N, 5 mL) was added, and the mixture was autoclaved (121 °C, 15 min.), cooled, adjusted to pH 7.0 with concentrated NaOH, saturated with NaCl, filtered, and extracted continuously with ether for 24 h. After evaporation of the extract, pantolactone (8 mg, 1.7 × 10^5 cpm, 2.8% radiochemical yield) was isolated by preparative TLC. It was thus calculated that 0.00478 mmol of pantoate had been biosynthesized from the exogenous valine. The incorporation of [4-^{13}C]-(2*RS*,3*S*)-valine into pantothenate and isolation of pantolactone was carried out in exactly the same manner as described for [^{14}C]valine.
(9) Medium I (g/L): K_2HPO_4 (7), KH_2PO_4 (3), sodium citrate (1.5), $MgSO_4 \cdot 7H_2O$ (0.10), $(NH_4)_2SO_4$ (1.0), $CaCl_2 \cdot 2H_2O$ (0.010), $FeSO_4 \cdot 7H_2O$ (0.0005), DL-valine (0.10), L-isoleucine (0.10), glucose (sterilized separately) (2), thiamine hydrochloride (sterilized separately) (0.0001), tap water to volume. Medium II: as in medium I, with omission of $(NH_4)_2SO_4$ and DL-valine and addition of β-alanine, 0.79 g/L (0.01 M).
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Vibrational Spectroscopy of the Electronically Excited State: Pulse Radiolysis/Time-Resolved Resonance Raman Study of Triplet β-Carotene

Sir:

One of the foremost problems in photophysics, photochemistry, and photobiology is adequate characterization of the structures of molecules in electronically excited states. This problem is particularly acute in solution, owing to short excited-state lifetimes and the inapplicability or lack of structural specificity of conventional (gas phase) excited-state probes. Recently, several workers have developed time-resolved resonance Raman (TR^3) techniques,[1-8] which meet the criteria of speed, sensitivity, and structural specificity[9] to be attractive probes for excited states. Yet with one exception[10] TR^3 has only been applied to ground-state transients. We report a resonance Raman study of the lowest triplet excited state of the photosynthetic accessory pigment, *all-trans*-β-carotene (see Figure 1).

Ground-state β-carotene gives a remarkably intense resonance Raman spectrum,[11] with bands which have been assigned to the in-phase, double-bond C=C stretch (1521 cm^{-1} in benzene), the C—C in-phase single-bond stretch (1157 cm^{-1}) and the C—H in-plane bend (1003 cm^{-1}).[11] These assignments are, however, recognized to be uncertain in the single-bond region.[11,12] The triplet state of β-carotene has been studied spectrophotometrically, using pulse radiolysis[13] as well as flash photolysis of chloroplasts.[14] In our experiment, a 10^{-4} M solution of β-carotene in benzene, containing 10^{-2} M naphthalene to transfer benzene triplets to β-carotene,[16] was irradiated by 4-MeV electron beam pulses of 800-ns duration. Transient absorption spectra (T-T λ_{max} 515 nm compared with ground-state λ_{max} of 460 nm) showed that the maximum concentration of β-carotene triplet states occurred at ~1 μs after the end of this radiolysis pulse (see Figure 2). The laser interrogation pulse (7-ns, 531.8-nm frequency-doubled Nd:YAG) was synchronized with the electron beam to strike the sample when the triplet concentration was near this maximum. The Raman photons were detected using a vidicon spectro-

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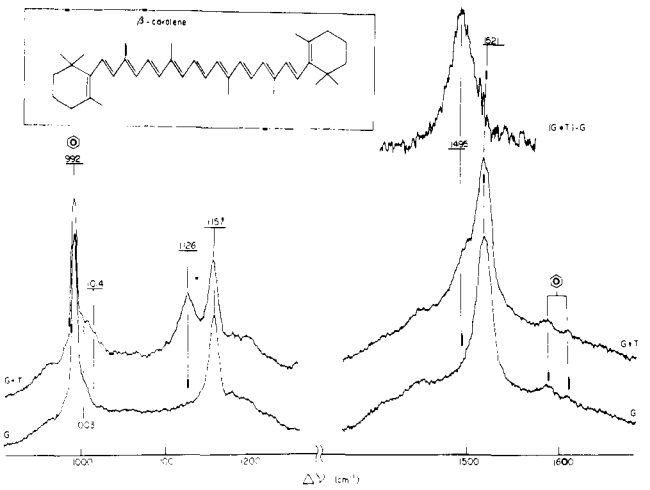

Figure 1. Lower trace (G): ground-state resonance Raman spectrum of β-carotene, excited by 7-ns pulses at the Nd:YAG second harmonic (531.8 nm, pulse repetition frequency 6 Hz, equivalent CW laser power 30 mW, signal accumulation time 53 s, solution conditions as stated in text); the benzene solvent peaks are denoted by hexagons. Middle trace (G + T): ground-state plus triplet-state resonance Raman spectrum of β-carotene; conditions are identical with ground-state spectrum except that the solution was exposed to the radiolysis pulse 2 μs prior to each laser pulse, promoting a fraction (~10% at the time of the laser pulse) of the β-carotene molecules to the lowest triplet state. Upper trace (G + T) − G: resonance Raman difference spectrum, showing the position of the triplet peak which appears as a shoulder on the ground-state peak in the (G + T) spectrum. Inset: carbon atom skeletal structure of β-carotene.

graph, which has recently been described in detail.[8] The only significant change in the apparatus for the present study was the use of detector gating electronics to reject the Cerenkov radiation which is produced synchronously with the radiolysis pulse (see Figure 2).

The results are shown in Figure 1. The lower trace in each of the spectrograph frames is the TR[3] spectrum of ground-state β-carotene. Comparison with CW-excited spectra shows that the ground-state spectrum is not perturbed by our pulsed-laser excitation conditions. The upper traces were recorded under the same conditions as the lower traces, except that the radiolysis pulse was delivered as shown in Figure 2. The ground-state features are still seen, and a new set of bands at 1495, 1126, and 1014 cm^{-1} arise. These are vibrations of the triplet state, which is responsible for the transient absorbance in Figure 2. The frequency shifts in the three major vibrations between the ground and the triplet states are listed in Table I. Additionally, the line widths of the triplet peaks are 1.3–1.4 times broader than those of the ground state.

The formation of the β-carotene triplet formally involves promotion of a π-bonding electron to a π-antibonding orbital as well as the obvious spin-state change. Calculations predict[11d,15] that the conjugation in polyene excited states is increased compared with that in the ground state. Each of these effects will tend to lower the frequency of the C=C double-bond stretch. Other calculations[17] suggest that, in β-carotene, the unpaired electrons of the triplet occupy orbitals primarily localized at two points in the polyene chain. This should lead to multiple peaks in the C=C region and a general increase in the C=C frequency. Our observation of a single C=C stretching frequency in the triplet, shifted 26 cm^{-1} to lower frequency than in the ground state, suggests delocalization of the triplet throughout the conjugated system.

Interpretation of the observed shifts of the vibrations in the single-bond region is complicated by uncertainties in the assignments of the ground-state normal modes. Additionally, the potential energy distribution of these modes may differ between the ground and triplet states. Assuming, however, that the potential energy distribution is similar in the ground state

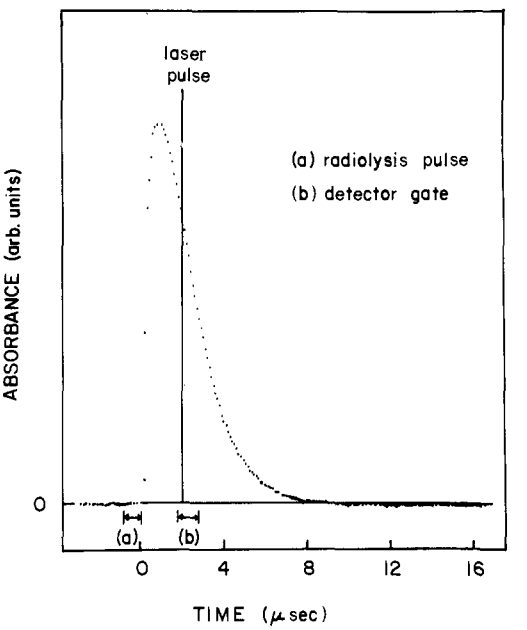

Figure 2. Transient absorbance at 525 nm due to β-carotene triplet concentration following pulse radiolysis. The temporal relationship among the radiolysis pulse, the laser pulse, and the detector gating is shown. The zero on the time axis is taken as the radiolysis pulse. See text for conditions and discussion.

Table I. Raman Frequencies and Line Widths[a] in the β-Carotene Ground and Triplet States

ground state		triplet state		frequency
frequency	line width[b]	frequency	line width	shift, G → T
1521	22	1495	31	−26
1127	13	1126	17	−31
1003		1014		+11

[a] In reciprocal centimeters. [b] Full width at half peak maximum.

and in the triplet,[15] the predicted[15,17] shortening of the C—C single bonds upon triplet formation requires that the C—C single-bond frequency *increase* in the triplet state. The data in Table I therefore suggest that the 1003-cm^{-1} (ground state) mode rather than the 1157-cm^{-1} mode is more accurately described as the C—C stretch. Alternatively, the C—C force constant may not change in the predicted manner.

The broader Raman peaks in the triplet state suggest a generally less rigid excited-state stereochemistry compared with that in the ground state. Detailed structural interpretation of the excited-state spectra will be aided by studies on structurally related systems. The present results demonstrate, however, that time-resolved resonance Raman spectroscopy shows excellent promise as a probe for the structures of excited-state molecules.

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Book Reviews

Crop Protection Agents—Their Biological Evaluation. Edited by N. R. McFARLANE (Shell Research Ltd). Academic Press. London. 1977. xvii + 638 pp. £19.50/$38.00.

This volume records the papers given at an International Conference on "The Evaluation of Biological Activity" held in Holland in 1975. The title is somewhat meaningless until coupled with the name of the organizers, The Pesticide Group of the British Society of Chemical Industry. It is also misleading in that two of the eight sessions dealt with plant growth regulators and chemicals controlling cattle ticks, which can hardly be termed "Crop Protection Agents".

However, most papers deal more or less with the main subject, techniques for evaluating biocides and bioregulators, and specifically with screening methods utilized in the discovery and development of insecticides, acaricides, fungicides, herbicides, insect and plant growth regulators, and chemicals affecting insect behavior. These contributions are obviously not intended as a catalogue of methods. Their purpose is rather to illustrate the diversity and relevance of the information required in this vast and fascinating field in which the ultimate objective is to increase food production by the discovery and introduction of new products, and techniques for their safe, effective use. Of course, all this leads to complication in handling and interpreting much data, necessitating the use of computer-based information systems and modelling techniques. Contributions on the latter and more general interface topics, such as pesticide legislation, effectively illustrate the technical, managerial, economic, social, and philosophical relevance of the methodology described.

The book will appeal to all who seek authoritative insight into a somewhat maligned technology, and particularly to those who are interested in its skills and disciplines. In the words of a plenary speaker who directs R&D in one of the largest and most successful commercial organizations developing pesticides, "the screening lab is absolutely not the place for second raters".

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Analysis with Ion-Selective Electrodes. By P. L. BAILEY (Electronic Instruments Limited). Heyden & Son Ltd., London. 1976. xii + 228 pp. $13.50.

The rapid development and increasing acceptance of potentiometric membrane electrodes has spawned a substantial number of books and monographs during the last few years. Such books generally fall into one of two categories, e.g., research monographs with multiple contributors drawn from active laboratories around the world and general volumes intended to survey the field for the nonspecialist. Bailey's book falls into the latter category with brief sections on theory, construction, selected properties, and analytical applications of typical ion-selective and gas-sensing membrane electrodes.

These topics are covered at a level acceptable for a novice to the field. The book will serve as a practical guide for the potential user of such membrane electrodes who desires to carry out routine analytical measurements or to "troubleshoot" commercial electrodes and associated products. The book also contains potentially useful compilations of selectivity data and of selected properties of the principal commercially available electrode types.

Unfortunately, Bailey's book does not cover any new ground and is similar to several other available volumes. The November 1976 publication date also means that the treatment lags behind present knowledge of the field, to some inevitable extent, although this is not a serious criticism as far as the practical side of the field is concerned.

There is some question in this reviewer's mind on whether it is still appropriate to deal with membrane electrodes, as has been done for some 12 years now, according to the nature of the membrane phase material. The classical distinction of membrane materials into the glass, liquid, and solid categories has become blurred in recent years. Such a division obscures the essential unity of phase boundary and selectivity considerations at the electrochemical and mechanistic levels apparent from much contemporary research. As a result, the book can be recommended only as a practical laboratory guide or as an introductory monograph.

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Physical Methods in Chemistry. By RUSSELL S. DRAGO (University of Illinois). Saunders, Philadelphia, Pa. 1977. xvi + 660 pp. $25.95.

This substantially enlarged revision of Drago's "Physical Methods in Inorganic Chemistry" is intended as a text for seniors and graduate students. The material covered includes symmetry and group theory (46 pp), MO theory (34 pp), electronic spectra (36 pp), vibrational and rotational spectra (55 pp), NMR (128 pp), EPR (43 pp), electronic, NMR, and EPR spectra of transition-metal complex ions (126 pp), magnetism (25 pp), NQR spectroscopy (20 pp), Mössbauer spectroscopy (22 pp), mass spectroscopy (14 pp), photoelectron spectroscopy (19 pp), and X-ray crystallography (38 pp). The development of NMR and EPR is much more thorough than that of vibrational and rotational spectroscopy; microwave spectroscopy receives only 2 pages, with no discussion of the rotational energy levels of polyatomic molecules. Discussions of instrumentation are brief. The emphasis is mainly, but not exclusively, on inorganic compounds.

The amount of theoretical material is much greater than in Drago's earlier book. Unfortunately, the theoretical discussions are generally poor. Many of the fundamental concepts are introduced without adequate definitions or explanations. Symbols are often used without definition. The theory is presented in a very fragmented form, with many equations and results poorly explained. There are a number of careless errors. (For example, the L_z eigenvalues are given as $0, 1, 2, \ldots, n-1$ and the electronic Hamiltonian of H_2 is written with a kinetic-energy operator for only one electron.)

The book gives very good specific examples of the applications of spectroscopy to lots of molecules, and this is its major strength. However, students will find the theoretical discussions more confusing than enlightening.

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