

consistent with the assigned structure; the observed chemical shifts and coupling constants were also in agreement with the NMR data reported by Tierney et al.¹³ following completion of these studies. The H₁ signal appeared at low field consistent with its assigned bay region location. The H₂ proton was found as a doublet of doublets at δ 6.13 coupled to H₁ and H₃ ($J_{1,2} = 9.5$, $J_{2,3} \approx 2.2$ Hz). The two carbinol H₃ and H₄ peaks overlapped at δ 4.60 and 4.69, respectively. The relatively large value of $J_{3,4}$ ($J_{2,3} = 2.3$, $J_{3,4} = 11.5$ Hz) confirms the trans stereochemical relationship of the hydroxyl groups and indicates that this dihydrodiol exists in solution predominantly in the trans-diequatorial conformation;²⁸ much smaller coupling constants are expected for the cis isomer or for the trans-diaxial conformation.^{7d,28}

Investigation of the carcinogenic activity of **2** has revealed it to be *more potent than DMBA and the most carcinogenic hydrocarbon metabolite tested to date*.²⁹ In comparison with the 5,6- and 8,9-dihydrodiols of DMBA, **2** (100 nmol) was found to induce tumors in 29/29 surviving mice (22.8 papillomas/mouse), while the other two dihydrodiols were essentially inactive. At lower dosage (10 nmol) **2** still induced tumors in 100% of mice (15.2 papillomas/mouse), whereas DMBA gave 85% tumor induction (4.8 papillomas/mouse). This is strong evidence for the intermediacy of **2** as a proximate carcinogenic metabolite and **1a** (and/or **1b**) as the ultimate carcinogenic form of DMBA.

The generality of the synthetic method depicted in Scheme I is supported by studies in progress aimed at extension of the method to the analogous dihydrodiols of other polycyclic arenes. *trans*-3,4-Dihydroxy-3,4-dihydro-7-methyl-BA, implicated as the proximate carcinogenic form of 7-methyl-BA,³⁰ has been synthesized successfully in our laboratory via a related synthetic sequence starting with 7-methyl-BA and eliminating the steps **6** \rightarrow **7** \rightarrow **8** involved with introduction of the methyl groups. Full details of this and other related syntheses will be reported in due course.

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Note Added in Proof. Since submission of this manuscript **2** has been characterized as a metabolite of DMBA by S. K. Yang, M. W. Chou, and P. P. Roller, *J. Am. Chem. Soc.*, **79**, 237 (1979); NMR data reported therein are in good agreement with those observed for authentic **2** obtained through synthesis.

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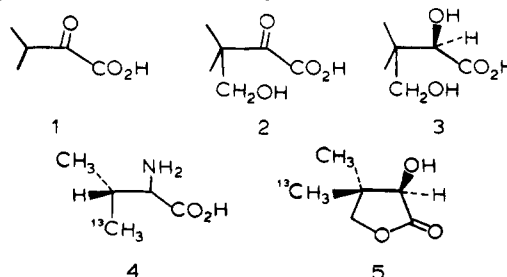
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Stereochemistry of Pantoate Biosynthesis from 2-Ketoisovalerate

Sir:

The biosynthesis of D-pantoate **3** constitutes the first stage in the formation of pantothenate, which is ultimately utilized in the biosynthesis of the acyl group carriers, coenzyme A and acyl carrier protein.¹ The first step in pantoate biosynthesis is the reaction of 2-ketoisovalerate **1** with *N*⁵,*N*¹⁰-methyl-enetetrahydrofolate to yield 2-ketopantoate **2**. The enzyme, ketopantoate hydroxymethyltransferase (5,10-methylenetetrahydrofolate: α -ketoisovalerate hydroxymethyltransferase), was recently isolated from *E. coli* and characterized by Snell and co-workers.² Subsequently, 2-ketopantoate is reduced by 2-ketopantoate reductase³ to D-pantoate **3**. I now present ev-



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 [4-¹³C]- (2*RS*,3*S*)valine⁴ (4, 20 mg, 0.17 mmol, 90 atom % ¹³C) 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 H₂SO₄), pantolactone **5** (9 mg) was isolated. The ¹³C 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 biosynthesized 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 ¹³C 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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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 (TR³) techniques,¹⁻⁸ which meet the criteria of speed, sensitivity, and structural specificity⁹ to be attractive probes for excited states. Yet with one exception¹⁰ TR³ 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 radiolysis¹³ 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-