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 H_1 signal appeared at low field consistent with its assigned bay region location. The H_2 proton was found as a doublet of doublets at δ 6.13 coupled to H_1 and H_3 ($J_{1,2}$ = 9.5, $J_{2,3} \simeq$ 2.2 Hz). The two carbinol H_3 and H_4 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** → **7** → **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.

References and Notes

(1) C. B. Huggins, J. Pataki, and R. G. Harvey, *Proc. Natl. Acad. Sci. U.S.A.*, **58**, 2253 (1967).
(2) "Survey of Compounds which have been Tested for Carcinogenic Activity", Public Health Service Publication No. 14, Superintendent of Documents, Washington, D.C., 1972–1973.
(3) R. C. Moschel, W. M. Baird, and A. Dipple, *Biochem. Biophys. Res. Commun.*, **76**, 1092 (1977).
(4) A. Dipple and J. A. Nebzydoski, *Chem.-Biol. Interact.*, **20**, 17 (1978).
(5) V. Ivanovic, N. E. Geacintov, A. M. Jeffrey, P. P. Fu, R. G. Harvey, and I. B. Weinstein, *Cancer Lett.*, **4**, 131 (1978).
(6) While the evidence cannot distinguish 1a from the isomeric 1,2-diol 3,4-epoxide, other studies have implicated bay region diol epoxides structurally related to 1a as the principal active forms of other carcinogenic hydrocarbons.[7] A theoretical basis for this finding has been proposed.[8] Exceptions to this theoretical prediction have also been noted.[9,10]
(7) Reviews: (a) R. G. Harvey and P. P. Fu in "Polycyclic Hydrocarbons and Cancer: Environment, Chemistry, and Metabolism", Vol. I, H. V. Gelboin and P. O. P. Ts'o, Eds., Academic Press, New York, 1978, Chapter 6, p 133; (b) W. Levin, A. W. Wood, P. G. Wislocki, R. L. Chang, J. Kapitulnik, H. D. Mah, H. Yagi, D. M. Jerina, and A. H. Conney, ref 7a, Chapter 9, p 189; (c) S. K. Yang, J. Deutsch, and H. V. Gelboin, ref 7a, Chapter 10, p 205; (d) for more recent references, cf. R. G. Harvey, H. M. Lee, and N. Shyamasundar, *J. Org. Chem.*, **44**, 78 (1979).
(8) D. M. Jerina, R. E. Lehr, H. Yagi, O. Hernandez, P. M. Dansette, P. G. Wislocki, A. W. Wood, R. L. Chang, W. Levin, and A. H. Conney in "In Vitro Metabolic Activation in Mutagenesis Testing", F. J. deSerres, J. R. Fonts, J. R. Bend, and R. W. Philpot, Eds., North-Holland, Amsterdam, 1976, p 159.
(9) R. G. Harvey and F. B. Dunne, *Nature (London)*, **273**, 566 (1978).
(10) P. P. Fu and R. G. Harvey, *Tetrahedron*, **34**, 857 (1978).
(11) C. Malaveille, H. Bartsch, B. Tierney, P. L. Grover, and P. Sims, *Biochem. Biophys. Res. Commun.*, **83**, 1468 (1978).
(12) R. G. Harvey, P. P. Fu, C. Cortez, and J. Pataki, *Tetrahedron Lett.*, 3533 (1977).
(13) Oxidation of DMBA by ascorbic acid–ferrous sulfate–EDTA afforded complex products from which **2** could be isolated by LC in <0.1% yield: B. Tierney, A. Hewer, A. D. MacNicoll, P. G. Gervasi, H. Rattle, C. Walsh, P. L. Grover, and P. Sims, *Chem.-Biol. Interact.*, **23**, 247 (1978).
(14) R. G. Harvey and K. Urberg, *J. Org. Chem.*, **33**, 2206 (1968); reduction of 13.7 g of BA gave 11.2 g of **3**.
(15) H. C. Brown, "Hydroboration", W. A. Benjamin, New York, 1962; H. C. Brown, "Boranes in Organic Chemistry", Cornell University Press, Ithaca, N.Y., 1972.
(16) Good yields of pure compounds were obtained at each stage and all new compounds (except **4a,b**) gave satisfactory C and H analysis within ±0.3% of theory; the mixed alcohols **4a,b** were utilized directly without microanalysis. NMR spectra were obtained on all compounds and were in good agreement with the assigned structures.
(17) K. Omura, A. K. Sharma, and D. Swern, *J. Org. Chem.*, **41**, 957 (1976).
(18) **6c:** mp 159–160 °C (mp 161–162 °C reported by D. C. C. Smith, *J. Chem. Soc.*, 673 (1962)).
(19) **6a:** mp 156–157 °C (mp 156.5–158.5 °C reported by L. Fieser and M. Fieser, *J. Am. Chem. Soc.*, **55**, 3342 (1933), and G. M. Badger, *J. Chem. Soc.*, 940 (1947)).
(20) Yields of **3, 4, 5,** and **6a,c** were 78, 60, 74, and 88%, respectively, for an overall yield of 31%.
(21) B. I. Rosen and W. P. Weber, *J. Org. Chem.*, **42**, 3463 (1977).
(22) M. S. Newman and V. Sankaran, *Tetrahedron Lett.*, 2067 (1977).
(23) **8a:** mp 130 °C (mp 131–132 °C reported by M. S. Newman, J. S. Khanna, K. Kanakarajan, and S. Kumar, *J. Org. Chem.*, **43**, 2553 (1978)).
(24) T. R. Kelly, H. M. Dali, and W. G. Tsang, *Tetrahedron Lett.*, 3859 (1977).
(25) D. H. R. Barton, A. G. Brewster, S. V. Ley, and M. N. Rosenfeld, *J. Chem. Soc., Chem. Commun.*, 985 (1976); D. H. R. Barton, S. V. Ley, P. D. Magnus, and M. N. Rosenfeld, *J. Chem. Soc., Perkin Trans. 1*, 567 (1977).
(26) E. Clar, "Polycyclic Hydrocarbons", Vol. I and II. Academic Press, New York, 1964.
(27) D. M. Jerina, H. Selander, H. Yagi, M. C. Wells, J. F. Davey, V. Mahadevan, and D. T. Gibson, *J. Am. Chem. Soc.*, **98**, 5988 (1976).
(28) R. E. Lehr, M. Schaefer-Ridder, and D. M. Jerina, *J. Org. Chem.*, **42**, 736 (1977); D. Zacharias, J. P. Glusker, P. P. Fu, and R. G. Harvey, *J. Am. Chem. Soc.*, in press.
(29) T. Slaga, G. L. Gleason, J. DiGiovanni, M. R. Juchau, K. B. Sukumaran, and R. G. Harvey, *Cancer Res.*, in press.
(30) C. Malaveille, B. Tierney, P. L. Grover, P. Sims, and H. Bartsch, *Biochem. Biophys. Res. Commun.*, **75**, 427 (1977).

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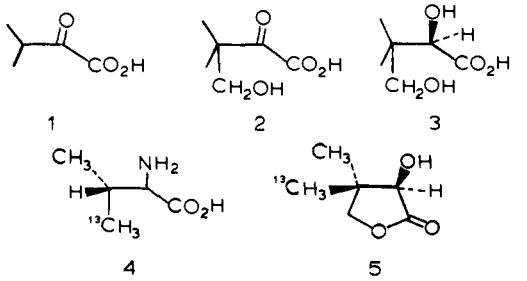
Stereochemistry of Pantoate Biosynthesis from 2-Ketoisovalerate

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}-methylenetetrahydrofolate 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 reductase[3] to D-pantoate **3.** I now present ev-



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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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References and Notes

(1) G. M. Brown in "Comprehensive Biochemistry", Vol. 21, M. Florkin and E. H. Stotz, Eds., American Elsevier, New York, 1971, pp 73–80.
(2) J. H. Teller, S. G. Powers, and E. E. Snell, *J. Biol. Chem.*, **251**, 3780–3785 (1976); S. G. Powers and E. E. Snell, *ibid.*, **251**, 3786–3793 (1976).
(3) H. L. King and D. R. Wilken, *J. Biol. Chem.*, **247**, 4096–4098 (1972); D. R. Wilken and R. E. Dyer, *Arch. Biochem. Biophys.*, **189**, 251–255 (1978).
(4) D. J. Aberhart and L. J. Lin, *J. Chem. Soc., Perkin Trans. 1*, 2320–2326 (1974); D. J. Aberhart, *Tetrahedron*, **33**, 1545–1559 (1977).
(5) J. H. Teller, Ph.D. Dissertation, University of California, Berkeley, 1970, pp 22, 57–59.
(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.
(7) R. R. Martin, V. D. Marshall, J. R. Sokatch, and L. Unger, *J. Bacteriol.*, **115**, 198–204 (1973); J. E. Norton and J. R. Sokatch, *Biochim. Biophys. Acta*, **206**, 261–269 (1970).
(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).
(10) J. B. Jones in "Applications of Biochemical Systems in Organic Chemistry", J. B. Jones, C. J. Sih, and D. Perlman, Eds., Wiley-Interscience, New York, 1976, pp 479–490.
(11) A. Grussner, M. Gatzi-Fichter, T. Reichstein, and K. Pfaltz, *Helv. Chim. Acta*, **23**, 1276–1286 (1940).
(12) N. S. Bhacca and D. H. Williams, "Applications of NMR Spectroscopy in Organic Chemistry", Holden-Day, San Francisco, 1964, p 190; H. Fritz and W. Lowe, *Angew. Chem., Int. Ed. Engl.*, **1**, 592–593 (1962).
(13) D. J. Aberhart, *Bioorg. Chem.*, **6**, 191–201 (1977).
(14) Hill and co-workers have enzymatically converted chirally labeled α,β-dihydroxyisovalerate to chirally labeled 2-ketoisovalerate, which was converted enzymatically to chiral valine without randomization of the labeling: R. K. Hill, S. Yan, and S. M. Arfin, *J. Am. Chem. Soc.*, **95**, 7857–7859 (1973).
(15) M. Akhtar and P. M. Jordan, *Tetrahedron Lett.*, 875–879 (1969).

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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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