previously.[3] Oxygen-18 analyses indicated that the alkyl-oxygen of the acid phthalate derivative had 3.60 atom % O^{18} excess (assuming discrete labeling) and that the pure α-phenylallyl alcohol-O^{18} had 3.54 atom % O^{18} excess.[18] Thus the purification process resulted in a loss of only *ca.* 1.4% of the oxygen-18 label. The labeled alcohol obtained by saponification of the acid phthalate derivative had b.p. 68.5–69° (0.15 mm.), $n^{25}D$ 1.5380; ultraviolet spectrum: shoulder, 248.5–249.5 mμ (ϵ 228), λ_{max} at 252.2, 258.0 and 264.0 mμ with ϵ 275, 287 and 213, respectively. This alcohol was redistilled twice before use in the exchange experiment.

Cinnamyl alcohol-O^{18} was prepared by the sodium borohydride reduction[19] of cinnamaldehyde-O^{18}. The cinnamaldehyde-O^{18} was prepared by equilibrating the unlabeled aldehyde with O^{18}-enriched water in the presence of a catalytic amount of *p*-toluenesulfonic acid. The cinnamyl alcohol-O^{18}, purified by recrystallization from ether–pentane, had m.p. 34.0–34.4°, $n^{25}D$ 1.5818 (supercooled liquid), and 0.54 atom % O^{18} excess; ultraviolet spectrum: λ_{max} at 251.0, 283.3 and 292.2 mμ with ϵ 17,600, 1200 and 843, respectively.

Oxygen-18 Exchange Experiments. Part A. Oxygen-18 Exchange Associated with the Rearrangement and Racemization of α-Phenylallyl Alcohol-O^{18}.—An acid ($HClO_4$) concentration of 0.0514 *M* and an initial $R\alpha O^{18}H$ concentration of 0.119 *M* were used in this experiment. The reaction solution was prepared by dissolving a weighed sample of labeled alcohol in 40% aqueous dioxane.[20] The reaction mixture also contained 0.0486 *M* $LiClO_4$. Thus the ionic strength was 0.1. After a short time for temperature equilibration a 94.10-ml. aliquot was withdrawn and delivered into a 100-ml. volumetric flask containing 5.00 ml. of 1 *M* aqueous NaOH to quench the reaction. Subsequent aliquots were withdrawn at appropriate time intervals and treated in the same manner. The quenched aliquots of reaction solution were diluted to exactly 100 ml. with distilled water and sampled (1-ml. aliquots) for ultraviolet spectral analysis (k_r determination, columns 2 and 3 of Table I). The remaining 99-ml. portions of quenched reaction solutions were saturated with sodium chloride and extracted with ether (150-, 100-, 50-ml. portions). The ethereal solutions of the binary mixture of α- and γ-phenylallyl alcohols were dried over K_2CO_3. The solvent was removed under reduced pressure and *ca.* 25% of the residue was distilled. The distillation flask was then transferred to a clean semi-micro distillation apparatus and the remainder of the material was distilled. The first few drops of distillate were discarded. The main fraction from this distillation was redistilled and three roughly equal fractions were collected. The third of these fractions was that used for ultraviolet spectral analysis and oxygen-18 determinations (columns 5 and 6, respectively, of Table I). This experiment is summarized in Table I. The method used to compute the O^{18} contents of the remaining α-phenylallyl alcohol (column 8, Table I) and the total phenylallyl alcohol in the reaction mixture (column 7) from the experimentally determined values for the isolated samples (column 6) has been described in a preceding section.

Part B. Oxygen-18 Exchange between Cinnamyl Alcohol-O^{18} and the Reaction Medium.—A solution of 0.12 *M* cinnamyl alcohol-O^{18} and 0.101 *M* $HClO_4$ was prepared and thermostated at 30° for 0, 5 and 10 half-periods for loss of optical activity of active $R\alpha OH$. The three samples of cinnamyl alcohol extracted from the quenched aliquots of reaction solution (isolated by distillation, and purified by recrystallization from ether–pentane) had the same melting points (34.2–34.6°) and oxygen-18 contents (0.54 atom % excess). The extinction coefficient for the absorption at 251 μ remained constant which shows that the concentration of $R\gamma OH$ did not vary during the experiment.

Isolation of Mixtures of α and γ-Phenylallyl Alcohol from Reaction Mixtures.—The following experiment was carried out to determine if the O^{18} contents of the isolated binary mixtures (column 6) and the O^{18} contents calculated from these experimental values (columns 7 and 8) were reliable. In effect this experiment is one in which known values are compared with experimentally determined values.

A mixture of *ca.* 75% α-phenylallyl alcohol-O^{18} (3.14 atom % excess) and 25% unlabeled cinnamyl alcohol was prepared. The O^{18} content of the binary mixture was 2.34 atom % O^{18} excess. This binary mixture was used to prepare a simulated reaction mixture. The solution (40% aqueous dioxane) also contained 0.1 *M* $LiClO_4$ and 0.003 *M* NaOH. A 1-ml. aliquot of this solution was withdrawn and shown to be 74.7% labeled α-isomer and 25.3% unlabeled α-isomer by ultraviolet analysis. From the composition and the O^{18} content of the α-isomer, the O^{18} content of the phenylallyl system in the reaction solution was calculated to be 2.34 atom % O^{18} excess.

The remainder of the reaction solution (99 ml.) was sampled (94.10 ml. aliquot) and a pure binary mixture of α- and γ-isomers (shown by infrared and ultraviolet spectra) was isolated as described in the kinetic experiment. The purified isolated sample consisted of 97.3% α-isomer and 2.7% γ-isomer (ultraviolet analysis) and had 3.05 atom % O^{18} excess. The calculated value of the O^{18} content of the phenylallyl alcohol in the reaction mixture (eq. 14 N_γ = 0) is 2.33 atom % O^{18} excess which is in excellent agreement with the known value (2.34 atom % O^{18} excess) obtained by direct analysis of the binary mixture used to make up the simulated reaction solution. Thus it appears that the isolation and analytical techniques employed in the kinetic experiment are reliable.

(19) S. W. Chaikin and W. G. Brown, THIS JOURNAL, **71**, 122 (1949).

(20) Prepared by mixing two volumes of pure dioxane and three volumes of conductivity water at 25°; see footnote 18, ref. 3.

COMMUNICATIONS TO THE EDITOR

STEREOCHEMISTRY OF TRISUBSTITUTED DOUBLE BONDS IN TERPENOIDS

Sir:

By the use of nuclear magnetic resonance spectroscopy, it has proved possible to determine readily for the first time the stereochemistry of certain trisubstituted double bonds bearing carbonyl substituents.[1–6] A variety of trisubstituted double bond more common in nature (found in many terpenoids) is the type $A\text{-}CH_2C(CH_3)=CHCH_2\text{-}B$; it has been stated[7] that n.m.r. cannot be used to distinguish geometrical isomers in one case of this sort. However, we have found relatively small (about 0.07 τ unit[8]) but very useful differences in the chemical shifts of the methyl hydrogens in the n.m.r. spectra of geometrical isomers for compounds with the types of double bonds shown in Table I.

(1) L. M. Jackman and R. H. Wiley, *Proc. Chem. Soc.*, 196 (1958).

(2) J. W. K. Burrell, L. M. Jackman and B. C. L. Weedon, *ibid.*, 263 (1959).

(3) M. D. Nair and R. Adams, THIS JOURNAL, **82**, 3786 (1960).

(4) D. E. Jones, *et al.*, *J. Chem. Soc.*, 2349 (1960).

(5) R. R. Fraser, *Can. J. Chem.*, **38**, 549 (1960).

(6) S. Fujiwara, *et al.*, *Bull. Chem. Soc. Japan*, **33**, 428 (1960).

(7) R. H. Wiley and L. M. Jackman in L. M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, Inc., New York, N. Y., 1959, pp. 121, 124.

(8) G. V. D. Tiers, *J. Phys. Chem.*, **62**, 1151 (1958).

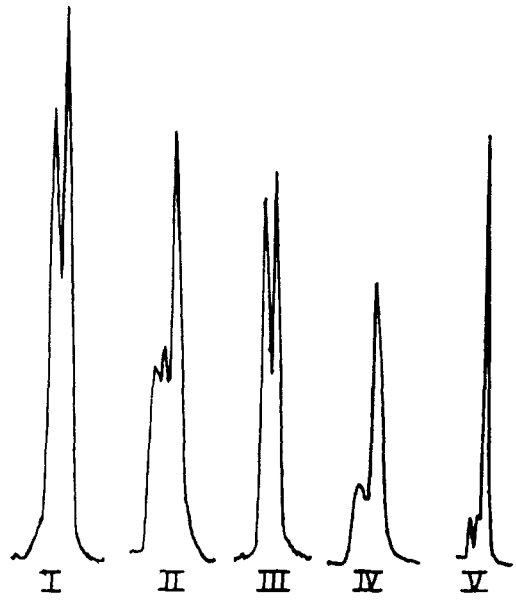


Fig. 1.—Methyl proton region of the n.m.r. spectra of some terpenoids: compounds, peak locations from left to right in τ units, and, in brackets, the types of double bonds responsible for the absorption (see Table I) are: I, *trans-trans*-farnesol, 8.34 [(5) *cis* and (4)], 8.405 [(5) *trans* and (2)]; II, *cis-trans*-farnesol, 8.27 [(3)], 8.34 [(5) *cis*], 8.41 [(5) *trans* and (2)]; III, 3-methyl-2-buten-1-ol, 8.26 [(6) *cis*], 8.33 [(6) *trans*]; IV, all-*trans*-squalene, 8.335 [(5) *cis* twice], 8.405 [(2) four times and (5) *trans* twice]; V, coenzyme Q_{10}, 8.28 [side-chain double bond nearest quinone ring], 8.35 [(5) *cis*], 8.42 [(5) *trans* once and (2) eight times].

The values in the table were first derived from these series of compounds; then, as more compounds with the various types of double bonds were run, slight changes were made in some cases to give better average values: (5) from 6-methyl-5-hepten-2-one; (1), (2), (3) and (4) from nerylacetone,[11] geranylacetone[11], nerol[2] and geraniol,[2] respectively, by subtracting the absorption of 6-methyl-5-hepten-2-one in the 8.0–8.5 region; (6) refers to 3-methyl-2-buten-1-ol, whose methyl proton absorption is shown in Fig. 1.[12] Also in agreement with these values are those for *hevea* (*cis*) and *balata* (*trans*) rubber, 8.33 (type (1)) and 8.40 (type (2)), respectively, and those for all-*trans*-squalene (Fig. 1).

These values provide a basis for assigning configurations to the farnesols,[11] since the four stereoisomers should have different patterns in the methyl proton region of their n.m.r. spectra. The critical regions for farnesols assigned the *trans-trans* and *cis-trans* configurations on chemical grounds[11] are shown in Fig. 1; the spectra verify these assignments.

Analysis of the n.m.r. spectrum of coenzyme Q_{10}[13] (Fig. 1) clearly indicates that eight of the double bonds in the side-chain have the *trans* configuration. The methoxyl groups and the methyl attached to the quinone ring have been shown to absorb below 8.1.[13] The methyl absorbing at 8.35 must be the one of type (5) *cis*, and of the eight at 8.42, one is of type (5) *trans*, leaving seven of type (2). The peak at 8.28 must correspond to the side-chain methyl group nearest to the quinone ring; if suitable model compounds were available, it would very likely be possible to learn the configuration of this double bond from the n.m.r. spectrum.

The values in the table hold well for the acyclic compounds considered so far. The dangers of extrapolating such n.m.r. data to cyclic systems have been emphasized[14]; nevertheless, caryophyllene[15] and isocaryophyllene,[15] which have 9-membered rings containing double bonds of essentially types (2) and (1), respectively, have the τ values (8.405 and 8.33, respectively, in both CCl_4 and $CDCl_3$) that would be expected from the table.

We also have run sesquiterpenoids with 10-membered rings containing double bonds of unknown configuration. The τ values for methyl groups attached to the trisubstituted double bonds were 8.395 and 8.58 for germacrone,[16] 8.32 and 8.59 for costunolide.[17] The high values

TABLE I[9]

τ VALUES OF METHYL PROTONS IN COMPOUNDS WITH THE GROUPING A-$CH_2C(CH_3)$=$CHCH_2$-B

Double bond type	A	B	Relationship between proton and absorbing methyl group[10]	
1	Isoprene unit	Isoprene unit	*cis*	8.34
2	Isoprene unit	Isoprene unit	*trans*	8.41
3	Isoprene unit	OH	*cis*	8.27
4	Isoprene unit	OH	*trans*	8.35
5	H	Isoprene unit	*cis*	8.34
			trans	8.40
6	H	OH	*cis*	8.26
			trans	8.33

(9) All spectra were determined on a Varian 60 Mc. high resolution spectrometer. Compounds were run 10% in CCl_4 containing 3% *tert*-butyl alcohol and *ca.* 3% tetramethylsilane, except for coenzyme Q_{10} (a spectrum of this compound, run 8% in CCl_4 with no *tert*-butyl alcohol added, was kindly furnished by Dr. J. N. Shoolery), costunolide (7% in $CDCl_3$ containing 3% *tert*-butyl alcohol and 3% tetramethylsilane), and pyrethrosin (10% in the latter solvent mixture). The *tert*-butyl alcohol was used as a second internal standard to correct partially for the non-linearity of the sweep, allowing τ values accurate to ± 0.01 in the 8.2–8.5 region. The *tert*-butyl alcohol methyl protons absorb at 8.793 in CCl_4 (8.755 in $CDCl_3$); a correction factor was applied when the *tert*-butyl alcohol methyl proton peak did not come at this position. The values in this table have been found to hold for several compounds (*e.g.*, nerylacetone and caryophyllene) in which A or B is not an isoprene unit, but some other grouping starting with a methylene unit.

(10) For double bond type (5) the *cis* and *trans* assignments are based on analogy to (1) and (2); similarly, the assignments for (6) are based on analogy to (3) and (4). Whether or not these assignments should be reversed does not affect the conclusions below.

(11) R. B. Bates, D. M. Gale and P. P. Nicholas, unpublished results.

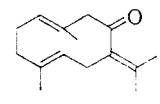
(12) It has been implied[7] that this compound has only one peak in the 8.3 region.

(13) K. Folkers, *et al.*, THIS JOURNAL, **80**, 4752, 4753 (1958).

(14) L. M. Jackman, ref. 1, p. 51.

(15) D. H. R. Barton, *et al.*, *J. Chem. Soc.*, 3124 (1953).

(16) F. Šorm, *et al.*, *Chem. and Ind.*, 1089 (1959); G. Ohloff, *et al.*, *Ann.*, **625**, 206 (1959). The germacrone n.m.r. spectrum fully supports the Šorm structure



and disallows the Ohloff structure. Key points are the presence of broad vinyl hydrogen peaks centered at 5.24 (relative area indicating two vinyl hydrogens) and the absence of peaks above 8.58 (indicating no angular methyl group and no cyclopropane hydrogens).

(8.58 and 8.59) for these compounds probably result from shielding of the absorbing methyl protons by the π electrons of the other ring double bond[18]; the occurrence of such shielding limits the number of configurations which must be considered for these compounds. It is noteworthy that this diamagnetic shielding is absent in pyrethrosin,[19] which exhibits absorption at 8.14; in this case, a paramagnetic shift derived from the attachment of an acetate function is observed.

We wish to express our sincere thanks to the Goodyear Tire and Rubber Co. and Dr. H. S. Gutowsky (rubber samples), Drs. F. Šorm and L. Dolejš (germacrone and costunolide), Dr. S. M. McElvain (caryophyllene), Dr. S. C. Bhattacharyya (costunolide), Dr. D. H. R. Barton (pyrethrosin), Mr. O. Norton (n.m.r. spectra), and the National Science Foundation (Undergraduate Fellowship to D.M.G.).

(17) V. Herout and F. Šorm, *Chem. and Ind.*, 1067 (1959); A. S. Rao, G. R. Kelkar and S. C. Bhattacharyya, *Tetrahedron*, **9**, 275 (1960).

(18) L. M. Jackman, ref. 1, p. 129.

(19) D. H. R. Barton, O. C. Böckman and P. de Mayo, *J. Chem. Soc.*, 2263 (1960).

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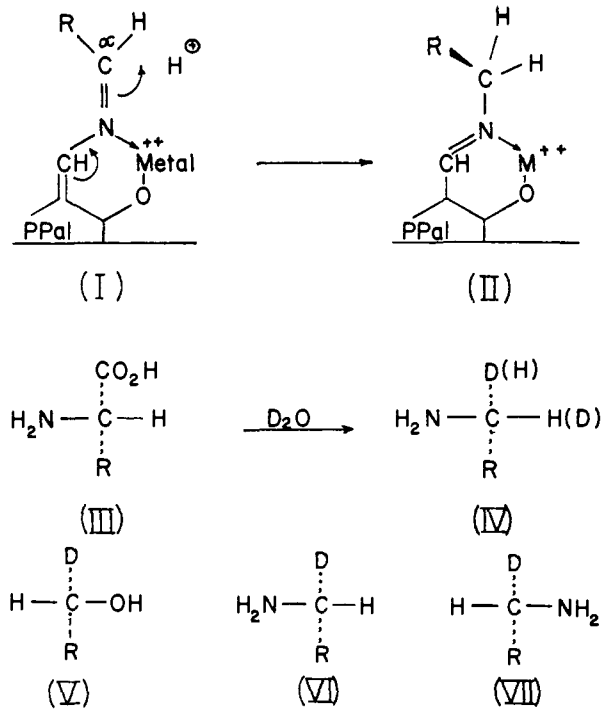
RECEIVED SEPTEMBER 21, 1960

THE STEREOCHEMISTRY OF THE ENZYMIC DECARBOXYLATION OF AMINO ACIDS

Sir:

The recently acquired strategic importance of asymmetrically deuterated biogenic amines in the field of pharmacology[1] and in mechanism studies with amine oxidases[2] has made it imperative to establish the absolute stereochemistry of the enzymic decarboxylation of amino acids. This ubiquitous biochemical reaction which is known to be pyridoxal phosphate (PPal)-dependent[3] is of practical value[2] in preparing optically pure α-deuterated amines. The work of Mandeles, Koppelman and Hanke[4] (in collaboration with F. Westheimer) has served to establish that tautomerization of the postulated Schiff base intermediate (I)[5] to give (II) must be stereospecific but it is not known whether the overall reaction proceeds with retention or inversion of configuration (III → IV). It might be expected, however, that the transition state for the release of carbon dioxide should resemble that for protonation of the α-carbon (I) since in all probability the same active site accommodates the R group (I) in both transition states. Accordingly, over-all retention of configuration may be expected in the enzymic decarboxylation of amino acids (III → IV). We



now wish to report unambiguous evidence in support of this view.

The chemical synthesis of both R- and S-α-*d*-tyramine[6] (VI and VII, respectively) from asymmetric intermediates of known absolute configuration has been accomplished. The relative rates of oxidation of these synthetic substrates by monoamine oxidase has allowed assignment of an absolute configuration to enzymically prepared α-*d*-tyramine.[2,4]

Hydroxylation of *p*-allylanisole (esdragol) with performic acid[7] gave 1,2-dihydroxy-3-(*p*-methoxyphenyl)-propane (b.p. 158°(2.5 mm.)), which was cleaved with lead tetraacetate in benzene to *p*-methoxyphenylacetaldehyde, b.p. 78–79° (0.1 mm.)[8] (65% over-all yield). Reduction of *d*-camphor with lithium aluminum deuteride in ether at −70° gave 1-*d*-isoborneol[9] at least 97% labeled on the carbinol carbon.[10] The deuterioisoborneol was converted to the bromomagnesium salt[11] and treated with *p*-methoxyphenylacetaldehyde according to Streitwieser's procedure.[12] There resulted a 40% yield of 1-*d*-*p*-methoxyphenethyl alcohol (V, R = *p*-methoxybenzyl), b.p. 95° (1 mm.)[13] $[\alpha]^{24}D$ −1.44° (neat).[14] Ac-

(1) B. Belleau, J. Burba, M. Pindell and J. Reiffenstein, forthcoming publication in *Science*.

(2) B. Belleau, M. Fang, J. Burba and J. Moran, THIS JOURNAL, **82**, 5752 (1960).

(3) A. E. Braunstein, "The Enzymes," Vol. 2, 2nd Ed., P. D. Boyer, H. Lardy and K. Myrbäck, Ed., Academic Press, New York, N. Y., 1960, p. 113.

(4) S. Mandeles, R. Koppelman and M. E. Hanke, *J. Biol. Chem.*, **209**, 327 (1954).

(5) D. E. Metzler, M. Ikawa and E. E. Snell, THIS JOURNAL, **76**, 648 (1954).

(6) Specification of asymmetric configuration according to R. S Cahn, C. K. Ingold and V. Prelog, *Experientia*, **12**, 81 (1956).

(7) D. Swern, "Organic Reactions," Roger Adams, Ed., John Wiley and Sons, Inc., New York, N. Y., 1953, Vol. VII, p. 378.

(8) H. Plieninger and B. Kiefer, *Chem. Ber.*, **90**, 617 (1957), reported the preparation of this aldehyde using different methods which proved unsatisfactory in our hands.

(9) A. Streitwieser, Jr., and W. D. Schaeffer, THIS JOURNAL, **79**, 6233 (1957).

(10) Determined by n.m.r. analysis. We are grateful to Dr. R. R. Fraser for the interpretation of the n.m.r. spectra and for stimulating discussions.

(11) G. Vavon and A. Antonini, *Compt. rend.*, **232**, 1120 (1951).

(12) A. Streitwieser, Jr., and J. R. Wolfe, Jr., THIS JOURNAL, **79**, 903 (1957).

(13) C. H. DePuy and R. E. Leary, *ibid.*, **79**, 3710 (1957).

(14) In a recent paper, Streitweiser[15] has noted that with the possible exception of enzymically prepared 1-*d*-ethanol, all optically