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Synthesis, Configuration, and Optical Purity of Asymmetric Primary Alcohols[1a]

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Samples of optically active and racemic isobutyl-1-d_1 alcohols required for the determination of the stereochemistry of the biosynthesis of the cholesterol side chain were prepared. Asymmetric reduction of isobutyraldehyde with (−)- and (+)-diisopinocampheyldeuterioboranes gave (−)-(1*R*)-isobutyl-1-d_1 alcohol (optical purity 27.1%) and (+)-(1*S*)-isobutyl-1-d_1 alcohol (optical purity 27.6%), respectively. Two specimens of optically pure (1*S*)-isobutyl-1-d_1 alcohol containing 80.5 and 70.2% deuterium were obtained by yeast reduction of samples of isobutyraldehyde-1-*d* containing 100 and 98.4% deuterium. The (+)-(3*R*)- and (−)-(3*S*)- isocaproic-3-d_1 acids, previously described by us, were shown to be 34.0 and 32.6% optically pure, respectively. The (+)-(1*S*)- and the (−)-(1*R*)-isobutyl-1-d_1 alcohol were related to (*R*)- and (*S*)-glyceraldehyde, respectively. The method of Horeau, studies utilizing the specificity of NAD^+ and yeast alcohol dehydrogenase (YADH), and a modified interpretation of the mode of reduction of ketones and aldehydes with (+)- and (−)-diisopinocampheylboranes led to identical configurational conclusions.

The transformation of lanosterol into cholesterol entails, among other steps, the reduction of the C-24 double bond. The stereochemistry of the addition of the hydrogen at C-24 is under investigation in our laboratory. The approach we chose was to biosynthesize cholesterol from 4(*R*)-2-^{14}C-mevalonic-4-t_1 acid (MVA) (t = ^{3}H), then cleave the side chain with an adrenal preparation, and isolate the resulting isocaproic acid. Ultimately, the stereochemistry at C-3 of the isocaproic acid, corresponding to that at C-24 of the cholesterol, would be established.[2]

Obviously the C-3 asymmetry of isocaproic-3-t_1 acid cannot be determined by relating it to a measurable rotation. An indirect approach was therefore required, and it was planned to degrade the acid to isobutyl-1-t_1 alcohol and define its configuration. The success of this approach depended heavily on two factors: our ability to degrade the isocaproic-3-t_1 acid without disturbing the asymmetry at C-3, and the feasibility of establishing the configuration at C-1 of the resulting alcohol. The stereospecific degradation of the acid to isobutyl-1-t_1 alcohol clearly was not an "insurmountable" problem, and we turned to the more involved question of determining the asymmetry at C-1 of the isobutyl-1-t_1 alcohol. At the outset, it was obvious that classical methods of defining the configuration of the isobutyl alcohol would be of no use, and that more specific microprocedures would be required. Under the circumstances, it appeared to us that the method of choice would be the NAD^+–alcohol dehydrogenase (ADH) oxidation of the alcohol to isobutyraldehyde. Because of the proven greater specificity of *yeast* alcohol dehydrogenase,[3] the studies were carried out with this enzyme.

Oxidation of ethanol with NAD^+ and *liver* ADH has been shown to proceed with the loss of the *pro*-(1*R*) proton.[4] The removal of the *pro*-(1*R*) proton was observed also on oxidation of geranyl-1-t_1 alcohol and farnesyl-1-t_1 alcohol with horse *liver* ADH.[5] In the reverse reaction, the enzymic reductions of 1-*d*-aldehydes, the (1*S*)-1-d_1-alcohols were obtained, indicating that the newly introduced hydrogen assumed the *pro*-(1*R*) orientation.[6] By analogy it seemed probable that NAD^+–YADH oxidation of isobutyl alcohol would also proceed with the removal of the *pro*-(1*R*) proton. Since this question was of paramount importance in our scheme of establishing the stereochemistry at C-24, prior to committing ourselves to this line of study we deemed it necessary to establish this point unequivocally.

As models for the studies, we required specimens of (+)- and (−)-isobutyl-1-d_1 alcohols of known absolute

(1) (a) This work was supported by Grants P-500H from the American Cancer Society and CA-K3-16614 from the National Institutes of Health; (b) Postdoctoral Fellow, 1966–1968.

(2) We have now proved that a 24-*pro*-(*S*) proton is added, resulting in the 24-(*R*) configuration [E. Caspi, K. R. Varma, and J. B. Greig, *Chem. Commun.*, 45 (1969)].

(3) See, for example, F. M. Dickinson and K. Dalziel, *Nature*, **214**, 31 (1967); *Biochem. J.*, **104**, 165 (1967).

(4) J. W. Cornforth, R. H. Cornforth, C. Donninger, G. Popjak, G. Ryback, and G. J. Schroepfer, *Proc. Roy. Soc.*, **163B**, 436 (1966), and references therein.

(5) C. Donninger and G. Popjak, *ibid.*, **163B**, 465 (1966); C. Donninger and G. Ryback, *Biochem. J.*, **11P**, 91 (1964).

(6) V. E. Althouse, D. M. Feigl, W. A. Sanderson, and H. S. Mosher, *J. Amer. Chem. Soc.*, **88**, 3595 (1966); W. A. Sanderson and H. S. Mosher, *ibid.*, **88**, 4185 (1966).

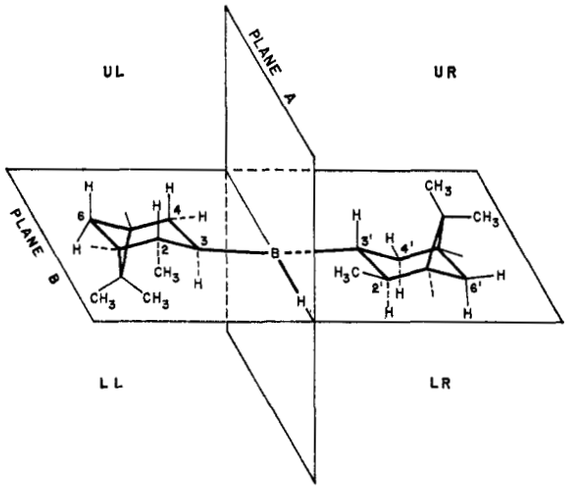


Figure 1.

stereochemistry. The optically active (+)- and (−)-alcohols were prepared by reduction of isobutyraldehyde with (+)- and (−)-diisopinocampheyldeuterioboranes, respectively.[7] The alcohols were purified through their respective acid phthalates, from which they were regenerated by treatment with lithium aluminum hydride. The acid phthalates were devoid of optical activity, but the recovered alcohols showed rotations: $[\alpha]^{20}D$ 0.168 ± 0.02° (neat), and $[\alpha]^{20}D$ −0.165 ± 0.02° (neat). Two additional specimens of alcohols were obtained by reduction of samples of isobutyraldehyde-1-*d* (98–100% deuterium) with fermenting yeasts.[6] Although the aldehyde was totally deuterated at C-1 (100% d_1), in one case the alcohol retained 80.5% *d*, $[\alpha]^{25}D$ 0.49° (neat, l = 1), and in the other case 70.2% *d*, $[\alpha]^{25}D$ 0.43° (neat, l = 1). There is ample evidence available demonstrating the stereospecificity of the reduction of aldehydes with fermenting yeasts, and it is implied that such alcohols are optically pure.[6] On the basis of this hypothesis, the rotation of the optically pure 100% C-1 monodeuterated isobutyl alcohol would be $[\alpha]^{20}D$ 0.61° (neat, l = 1). Presumably, in analogy to other cases,[6] this alcohol has the (1*S*) configuration. Consequently, the configurations and optical purities of the chemically synthesized alcohols would be (−)-(1*R*)-isobutyl-1-d_1 alcohol, 27.1%; and (+)-(1*S*)-isobutyl-1-d_1 alcohol, 27.6%.

Since the implied (1*S*) configuration of the isobutyl alcohol obtained by yeast reduction is of great importance in our considerations, we wished to corroborate it by other, more direct means. For evaluation of the configurational assignment, we had at our disposal three approaches: (a) Horeau's esterification procedure,[8] (b) deductions from the mode of reduction of aldehydes with (+)- and (−)-diisopinocampheyldeuterioboranes, and (c) degradation of the previously prepared (+)-(3*R*)-and (−)-(3*S*)-isocaproic-3-d_1 acids to the respective isobutyl alcohols, and comparison of their behavior toward NAD^+–YADH oxidation with those of the "synthetic" samples.

The Horeau method, though attractive and convenient for secondary alcohols, is of limited value when

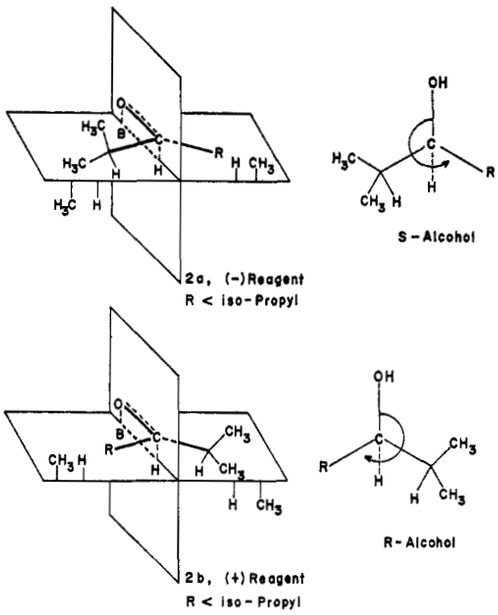


Figure 2.

applied to C-1 deuterated primary alcohols. Its drawback is the low, frequently marginal, optical activity observed for the recovered α-phenylbutyric acid in experiments with primary alcohols.[9] In our hands, with the use of a Hilger MK-III polarimeter, no meaningful readings of optical rotation were obtained for the α-phenylbutyric acid recovered from esterification of the chemically prepared (+)- and (−)-isobutyl-1-d_1 alcohols.

Brown, *et al.*, have devised a procedure for the asymmetric synthesis of alcohols through hydroboration of olefins and reduction of carbonyls with (+)- and (−)-diisopinocampheylboranes.[7] The same authors suggested a model for the mode of action of the reagent. The model was of limited utility since it was applicable only to the hydroboration of cyclic olefins, *cis* olefins, and terminal methylenes.

Other investigators[10,11] suggested alternative, frequently complicated, rationalizations. We had occasion to use the reagent for the preparation of (+)-(3*R*)- and (−)-(3*S*)-4-methylpentane-1,3-diol-1-tetrahydropyranyl ethers (**2** and **3**), by reduction of 4-methyl-3-ketopentane-1-ol-tetrahydropyranyl ether[12a] (**1**). The configurations of the products were determined by Horeau's method. To explain the configurations at C-3, we had to revise the mode of formation of the four-membered transition state.[12b] A schematic presentation of the disposition in space of groups of (−)-diisopinocampheylborane is given in Figure 1. The B–H bond is drawn to lie at the intersection of planes A and B, and bonds C_3–B–C'_3 are assumed to lie in plane B. Under these circumstances, the C_2 and C'_2 methyls of the (−) reagent will lie in the lower left (LL), and upper right (UR) quadrants (Figure 1). The opposite situation (not shown in Figure 1) will prevail in the (+) reagent, in which the C_2 and C'_2 methyls will be located

(7) H. C. Brown, N. R. Ayyangar, and G. Zweifel, *J. Amer. Chem. Soc.*, **86**, 397 (1964), and **86**, 1071 (1964); H. C. Brown and D. B. Bigley, *ibid.*, **83**, 3166 (1961).

(8) A. Horeau and B. Kagan, *Tetrahedron*, **20**, 2431 (1964), and references therein.

(9) A. Horeau and A. Nouaille, *Tetrahedron Lett.*, No. 33, 3953 (1966).

(10) A. Streitweiser, Jr., L. Verbit, and R. Bittman, *J. Org. Chem.*, **32**, 1530 (1967).

(11) D. R. Brown, S. F. A. Kettle, J. McKenna and J. M. McKenna, *Chem. Commun.*, 667 (1967).

(12) (a) E. Caspi and K. R. Varma, *J. Org. Chem.*, **33**, 2181 (1968); (b) K. R. Varma and E. Caspi, *Tetrahedron*, **24**, 6365 (1968).

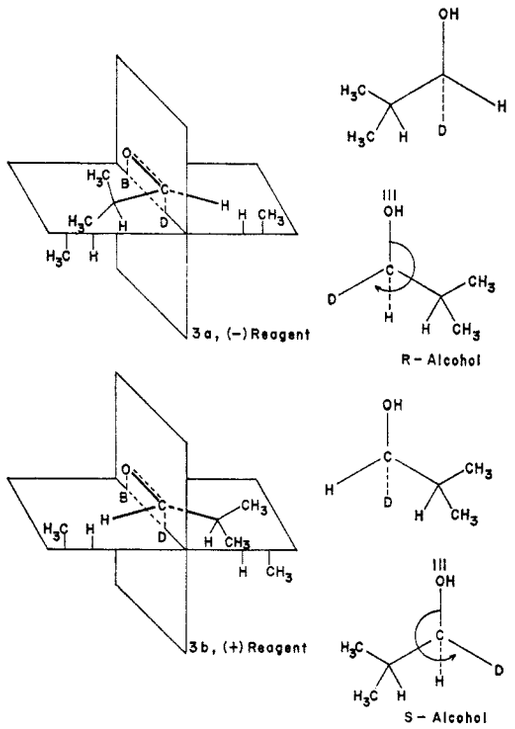


Figure 3.

in the upper left (UL) and lower right (LR) quadrants. It is evident that the carbonyl will approach the (−) reagent (Figure 2a) and the (+) reagent (Figure 2b) either from above or below the plane B, along plane A. For simplicity we have indicated in Figures 2a and b the formation of the four-membered transition states only *via* a top approach of the ketone to the reagents. The correct configurations of the produced alcohols can now be predicted effectively by assuming that in the actual reaction *the larger substituent* of the ketone is located in a *quadrant opposite to that which contains the* C_2 *and* C'_2 *methyls* of the reagent. Indeed, our results fully agreed with these predictions.[12]

We have reason to believe that this interpretation should also be applicable to the asymmetric reduction of isobutyraldehyde with (+)- and (−)-diisopinocampheyldeuterioboranes. Since the isopropyl group of the aldehyde is distinctly the larger substituent, the transition states of reactions with the (−) and (+) reagents can be represented as in Figures 3a and 3b. The anticipated result would therefore be the (+)-(1*S*)- isobutyl-1-d_1 alcohol from the (+)-*d* reagent (Figure 3b), and (−)-(1*R*)-isobutyl-1-d_1 alcohol from the (−)-*d* reagent (Figure 3a). We were encouraged in this interpretation of the reactions, and hence in the assignment of the configurations of the alcohols, by the fact that the isobutyl-1-d_1 alcohol obtained by yeast reduction of the 1-*d*-aldehyde, which was expected to have the (1*S*) configuration, indeed showed a positive rotation.

The correlation of the three specimens of the alcohols with the previously prepared (3*R*)- (**4**) and (3*S*)-isocaproic-3-d_1 acids (**5**)[12a] was now undertaken. The (+)-(3*R*)- and (−)-(3*S*)-acids were degraded to the corresponding alcohols **9** and **12** in the same manner. The (3*R*)-methyl ester **4a** (Figure 4) was first treated with phenylmagnesium bromide, then with acid to yield the olefin **6**. The olefin was ozonized, and after a reductive work-up (zinc–acetic acid) the isovaleraldehyde **7**

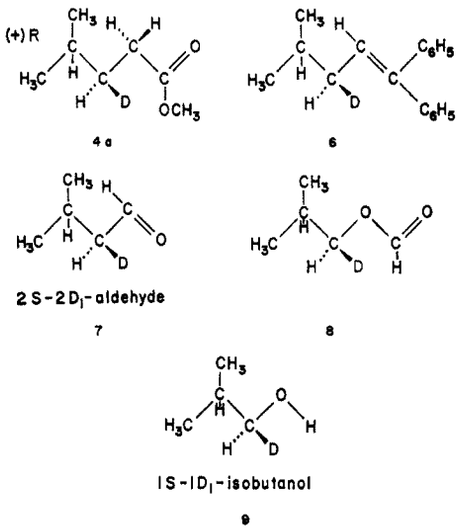
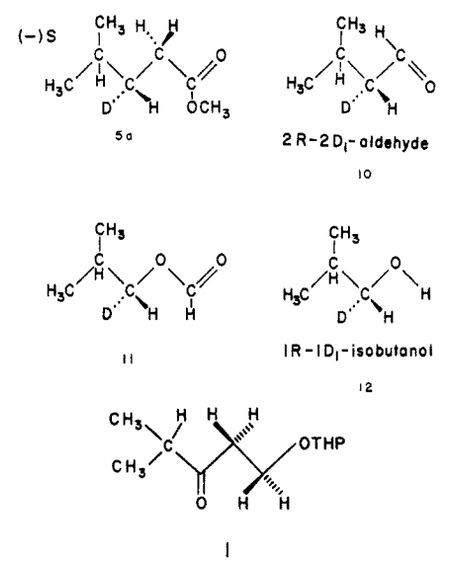


Figure 4.



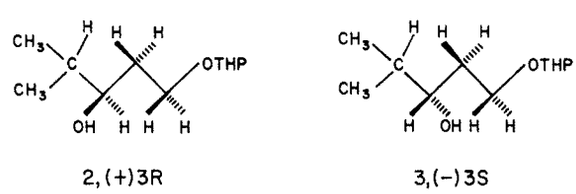


Figure 5.

was obtained. Because of the change of priorities of substituents at C-2 of the aldehyde, the (3*R*)-acid **4** gave the 2*S*-isovaleraldehyde-2-d_1 (**7**). The deuterated aldehyde **7** was treated with trifluoroperacetic acid, and the resulting formate **8** was reduced with lithium aluminum hydride to yield the (1*S*)-isobutyl-1-d_1 alcohol (**9**).[13a] The Baeyer–Villiger oxidation is known to proceed with retention of configuration.[13b] The (−)-(*S*) ester **5a** (Figure 5) was degraded in a similar manner *via* the aldehyde **10** and formate **11** to yield the (1*R*)-isobutyl-1-d_1 alcohol (**12**). Because of the availability of only small amounts of the alcohols so obtained, a comparison of their rotations with those of the previously described specimens was impossible. Consequently,

(13) (a) H. Weber, J. Seibl, and D. Arigoni, *Helv. Chim. Acta*, **49**, 741 (1966); (b) K. Mislow and J. Brenner, *J. Amer. Chem. Soc.*, **75**, 2318 (1953).

the correlation was accomplished by comparing their behavior toward NAD^+–YADH oxidation.

As mentioned above, in the several instances investigated until now, oxidation of primary alcohols to aldehydes with NAD^+–YADH proceeded with the removal of the *pro*-(1*R*) proton. As a working hypothesis, we assumed that the same would occur in the oxidation of the isobutyl-1-d_1 alcohols to isobutyraldehydes. Thus oxidation of the (1*S*)-isobutyl-1-d_1 alcohol would proceed by abstraction of the hydrogen, to yield isobutyraldehyde-1-*d*. On the other hand, oxidation of the (1*R*)-1-d_1 alcohol would require breakage of the C–D bond and formation of isobutyraldehyde. Involvement of a primary isotope effect could be anticipated in the breakage of the C–D bond and a secondary isotope effect in the scission of the C–H bond [of the (1*S*)-alcohol]. The isotope effects would not have presented a problem if the oxidation of the mixtures of enantiomeric, optically active (but not optically pure) alcohols could be brought to completion. Unfortunately, exploratory studies with 5–20-mg samples of isobutyl alcohol showed that the reaction is arrested when about 35–40% of alcohol is oxidized. In view of this, it was necessary to determine the magnitude of the isotope effects in the oxidation with NAD^+ and YADH.

Oxidation of samples of (1*S*)-isobutyl-1-d_1 alcohol containing 80.5% D and 70.2% D with NAD^+–YADH gave specimens of isobutyraldehyde-1-*d* having 78.7% D and 71.2% D, respectively. It is evident that the aldehydes retained all the deuterium, and this confirms the stereospecific removal of the *pro*-(1*R*) proton in the reaction. In addition, it is clear that the influence of a secondary isotope effect must be small, and its magnitude falls within the limits of mass spectroscopic deuterium determination. Consequently, for the present calculations we will disregard the secondary isotope effect.

In the absence of the optically pure (1*R*)-isobutyl-1-d_1 alcohol, the primary isotope effect could not be determined directly. Under the circumstances, we chose to define the primary isotope effect from the oxidation of racemic isobutyl-1-d_1 alcohol. The rationale of the approach was based on the assumption that the oxidation of the (1*S*)-1-d_1-alcohol will proceed at a different rate, probably faster, than that of the (1*R*)-1-d_1-alcohol. Should the two rates happen to be equal, the resulting aldehyde will retain 50% of the initially present deuterium. If, on the other hand, the oxidation of the (1*R*)-1-d_1-alcohol is slower, as expected, more than 50% of the initially present deuterium will be retained. From this excess (above 50%) the primary isotope effect X can be calculated.

The required (±)-isobutyl-1-d_1 alcohol (100% 1-d_1) was obtained by reduction of isobutyraldehyde with lithium aluminum deuteride. Oxidation of this alcohol proceeded to the extent of 40% within 20 hr, and gave 1-*d*-isobutyraldehyde containing 68% deuterium. For reasons discussed above it may be assumed that the (1*S*)-alcohol is oxidized at the "same rate" as the protonated isobutyl alcohol, and gives the 1-*d*-isobutyraldehyde. On the other hand, the (1*R*)-alcohol is oxidized at a rate X and gives the *h*-aldehyde. The primary isotope effect X (*d*/*h*) can now be calculated from eq 1,

$$\frac{(50)}{(50) + (50)\,(X)} \cdot 100 = 68 \qquad (1)$$

and therefore X = 0.47. This calculation will be valid as long as both enantiomeric alcohols are present in solution and are still available for oxidation. The magnitude of X = 0.47 indicates that, at any particular time, as long as both enantiomers are present in the medium, 2.13 molecules of the (1*S*)-alcohol will be oxidized for each molecule of the (1*R*)-alcohol.

Having evaluated the primary and secondary isotope effects, we turned to the question of confirmation of the configuration of the (−)-(1*R*)- and (+)-(1*S*)-isobutyl-1-d_1 alcohols prepared by reduction of isobutyraldehyde with (−)- and (+)-diisopinocampheyldeuterioboranes, respectively.

The (−)-(1*R*)-isobutyl-1-d_1 alcohol (100% 1-d_1) with an optical purity of 27.1% was oxidized in the standard manner (40% oxidation), and gave isobutyraldehyde containing 55.1% deuterium. The initial alcohol contained a 27.1% excess of the (*R*)-alcohol, and 72.9% racemate which consisted of 36.5% each of the (*R*)- and (*S*)-alcohols. Hence the actual composition of the sample was 63.5% (1*R*)-1-d_1-alcohol and 36.5% (1*S*)-1-d_1-alcohol. The anticipated amount of deuterium in the resulting aldehyde is given by eq 2, shown below,

$$\frac{(36.5)}{(36.5) + (63.5)\,(0.47)} \cdot 100 = Y \qquad (2)$$

and consequently Y = 55.0% deuterium. The calculated value for Y = 55.0% agrees well with the experimentally determined amount of deuterium present in the aldehyde (55.5%).

When a similar oxidation was performed on the (+)-(1*S*)-isobutyl-1-d_1 alcohol (100% 1-d_1) having an optical purity of *ca.* 27.6%, the isolated isobutyraldehyde retained 77.8% deuterium. The oxidized alcohol had an excess of 27.6% of the (1*S*) enantiomer, and the remaining racemate (72.4%) consisted of 36.2% each of the (1*S*)- and (1*R*)-alcohols. Consequently, the sample contained a total of 63.8% (1*S*)-isobutyl-1-d_1 alcohol and 36.2% (1*R*)-isobutyl-1-d_1 alcohol. The expected amount Z of deuterium in the aldehyde is given by eq 3,

$$\frac{(63.8)}{(63.8) + (36.2)\,(0.47)} \cdot 100 = Z \qquad (3)$$

where Z = 79.0% deuterium. The calculated (79.0%) and the experimentally found (77.8%) amount of deuterium in the aldehyde are in satisfactory agreement.

We now wished to correlate the above specimens of (−)-(1*R*)- and (+)-(1*S*)-1-d_1-alcohols with the (1*R*)- and (1*S*)-isobutyl-1-d_1 alcohols obtained by degradation of (−)-(3*S*)- and (+)-(3*R*)-isocaproic-3-d_1 acid, respectively. We have previously shown that the isocaproic-3-d_1 acids contained 11% D at the C-4 methine carbon.[12a] Consequently, the derived isobutyl alcohols, and hence isobutyraldehydes, should have 11% D at the methine carbons. Since the isobutyraldehydes are recovered as 2,4-DNPH derivatives from an acidified reaction medium, some deuterium may be lost by enolization. To evaluate the magnitude of the losses, we required isobutyl-2-*d* alcohol which was prepared as follows. Isobutyraldehyde was converted into isobutenyl acetate, and the enol ester was hydrolyzed in D_2O–D_2SO_4. The obtained $(CH_3)_2CD$–CHO (100% D) was diluted with protiated aldehyde and reduced with lithium aluminum hydride. A specimen of $(CH_3)_2CD \cdot CH_2OH$ (10.9% D) was treated with NAD^+–YADH, and

the resulting aldehyde was isolated in the usual manner. The aldehyde contained 8.1% D, indicating a net loss of 2.8% D from C-2. It follows that results for the isobutyraldehydes obtained by oxidation of isobutyl alcohols derived from the isocaproic acids should be increased by 2.8%.

The (1*R*)-isobutyl-1-d_1 alcohol from the (3*S*)-acid had 89.0% D at C-1 and 11% D at C-2, and on oxidation yielded isobutyraldehyde containing 55.0% deuterium. Thus the composition of the alcohol was 11% D at C-2, *A*% (1*R*)-1-d_1-alcohol and *B*% (1*S*)-1-d_1-alcohol. Obviously eq 4 obtains, and therefore, also, eq 5.

$$A + B + 11 = 100 \quad (4)$$

$$\frac{(11) + (B)}{(11) + (B) + (A)\,(0.47)} \cdot 100 = 57.8 \quad (5)$$

Solution of eq 4 and 5 leads to $B = 28.2\%$ and $A = 60.8\%$. It follows that the sample contained 28.2% (1*S*)-alcohol and 60.8% (1*R*)-alcohol. The optical purity of the (1*R*)-alcohol, and hence of the (3*S*)-isocaproic-3-d_1 acid, was 32.6% (60.8–28.2%).

The (1*S*)-1-d_1-alcohol derived from the (3*R*)-isocaproic-3-d_1 acid had 100% D, of which 11%[12a] was at C-2 and 89% at C-1. The composition of this sample was *A*% (1*R*)-1-d_1, *B*% (1*S*)-1-d_1, and 11% D at C-2. Oxidation of this specimen of (1*S*)-1-d_1-alcohol gave isobutyraldehyde which contained 82.1% deuterium. The over-all composition of the sample can be expressed by eq 4 and therefore by eq 6 which follows.

$$\frac{(B) + 11}{(B) + (11) + (0.47)\,(A)} \cdot 100 = 84.9 \quad (6)$$

Solution of eq 4 and 6 gives $A = 27.5\%$ and $B = 61.5\%$. Consequently, the optical purity of this specimen of (1*S*)-isobutyl-1-d_1 alcohol, and therefore of the (3*R*)-isocaproic-3-d_1 acid, was 34.0% (61.5–27.5%).

It is evident that the configurations of the (1*R*)- and (1*S*)-alcohols, obtained from the isocaproic acids, have been correctly assigned. As expected, oxidation of the (1*R*)-alcohol proceeded with a greater loss of deuterium than that of the (1*S*)-alcohol. This is in agreement with the anticipated removal of the *pro*-(1*R*) hydrogen (or of isotopic hydrogen) during the NAD^+ and YADH oxidation.[4] Furthermore, the results relate the configurations of (3*R*)- and (3*S*)-isocaproic-3-d_1 acids to those of the (1*S*)- and (1*R*)-isobutyl-1-d_1 alcohols. Since, in principle, the assignment of configurations of the acids was based on Horeau's procedure,[12a] and that of the isobutyl-1-d_1 alcohols on behavior toward NAD^+–YADH, it follows that both procedures lead to identical configurational conclusions. The results confirm also the configurations of the (−)-(1*R*)- and (+)-(1*S*)-isobutyl-1-d_1 alcohols synthesized by reduction of isobutyraldehyde with (−)- and (+)-diisopinocampheyldeuterioboranes. Thus our proposed interpretation of the "nature" of the transition state in the reduction of aldehydes and ketones with diisopinocampheylboranes gains added validity.

The (−)-4-methylpentane-1,3-(*S*)-diol was previously correlated with (*S*)-glyceraldehyde.[14,12a] The method of synthesis of the asymmetric isocaproic-3-d_1 acids[12a] from the asymmetric 4-methylpentane-1,3-diols proceeded with inversion of configuration at C-3. It follows that (+)-(3*R*)-isocaproic-3-d_1 acid (**4**) and the derived (2*S*)-2-d_1-aldehyde (**7**) and (+)-(1*S*)-isobutyl-1-d_1 alcohol (**9**) are related to (*R*)-glyceraldehyde. Similarly, the (−)-(3*S*)-isocaproic-3-d_1 acid (**5**) and the derived (2*R*)-2-d_1-aldehyde (**10**) and (−)-(1*R*)-isobutyl-1-d_1 alcohol (**12**) must be related to (*S*)-glyceraldehyde.

In summary, it may be concluded that the three methods, deductions on the reduction of ketones and aldehydes with (+)- or (−)-diisopinocampheylboranes, Horeau's procedure for secondary alcohols, and NAD^+–YADH oxidation, all gave analogous results and can be used for configurational assignments.

Experimental Section

Materials and Apparatus.—The sodium borodeuteride and lithium aluminum hydride were of high isotopic purity and were supplied by Metal Hydrides, Inc., Beverly, Mass. A solution of deuterioborane in tetrahydrofuran was prepared according to the general procedure used for diborane.[12a] The α-pinenes showed $[\alpha]^{25}D$ −45.5° and 46.0° (neat, $l = 1$), and were purchased from Chemical Samples Co., Columbus, and Aldrich Chemical Co., Milwaukee, respectively. The NAD^+ (85.5% β-NAD by enzymatic assay and 97.0% by uv assay) and crystalline yeast ADH (80%, specific activity 300 μ/mg) were used as supplied by Calbiochem Co., Los Angeles, Calif.

Analytical and preparative glc were carried out on an F & M Model 720 instrument on columns packed with TCEP, using helium as carrier gas. In all cases, identity of samples was confirmed by mixed injection with authentic (nondeuterated) samples. The melting points were determined on a hot stage and are corrected. The ir spectra were determined on a Perkin-Elmer Model 237 instrument. The mass spectra were run on a Varian M-66 or Hitachi–Perkin-Elmer RMH6 spectrometer. The nmr spectra were recorded at 60 Hz on a Varian DA-60 instrument using tetramethylsilane as internal standard. The deuterium content in all compounds was established mass spectroscopically. The uv spectra of the enzymatic oxidation media were recorded on a Perkin-Elmer Model 202 instrument in 1-cm cells. A Hilger polarimeter Model MK-III was used.

Isobutyraldehyde-1-*d*.—To a cooled stirred suspension of lithium aluminum deuteride (5.0 g, 119 mmol) in dry ether (100 ml), a solution of absolute ethanol (16.43 g, 357 mmol) in dry ether (50 ml) was added during 20 min. After stirring for another 20 min at 0°, isobutyronitrile (8.28 g, 120 mmol) was added during 5 min. The resulting thick solid was allowed to warm to room temperature and was stirred for 1.5 hr. The reaction was terminated with 5 *N* sulfuric acid, and the solid was removed by filtration and washed with ether. The combined filtrate and washings were concentrated by distillation through a 90-cm column packed with glass helices. The resulting concentrate was then distilled through a 1-ft packed column, and the fraction at bp 50–78° (750 mm) was collected. Analysis of this fraction by glpc at 60° on a 2.4-m column of 5% TCEP on Chromosorb indicated a yield of 5.06 g (60.6%) of isobutyraldehyde-1-*d*. The product was contaminated with large amounts of ethanol, but no other impurity was detected. Subsequently, this fraction was used for the preparation of (1*S*)-isobutyl-1-d_1 alcohol by yeast reduction (*vide infra*).

An aliquot of the aldehyde was converted into the 2,4-dinitrophenylhydrazone derivative, mp 183–186° (lit. 187 or 182° for nondeuterated material).[15] The mass spectrum of this derivative indicated that the aldehyde was 100% deuterated.

(+)-(1*S*)-Isobutyl-1-d_1 Alcohol. A. Reduction of Isobutyraldehyde-1-*d* with Fermenting Yeast.—In a 5-l. flask, dextrose (450 g) and distilled water (1.91 g) were stirred at 37° until completely dissolved. Baker's yeast (450 g, National Corporation) was added in small lumps with stirring. After a few minutes, a solution of isobutyraldehyde-1-*d* (2.53 g) in ethanol (10 ml) was added to the actively fermenting mixture. Active fermention slowed down considerably after 5 hr, but the reaction was continued with stirring for 12 hr (35–37°). The mixture was then steam distilled, and 1.3 l. of distillate was collected. The dis-

(14) G. Buchi, L. Crombie, P. J. Godin, J. S. Katlenbronn, K. S. Siddalingaiah, and D. A. Whiting, *J. Chem. Soc.*, 2843 (1961).

(15) M. Frankel and S. Patai, "Tables for Identification of Organic Compounds," Chemical Rubber Publishing Co., Cleveland, Ohio, 1960.

tillate was saturated with salt and continuously extracted with ether (12 hr). The ether was changed and the extraction was continued for 12 hr. The two ether extracts were combined and fractionated through a 90-cm packed column in order to remove the ether and most of the ethanol. The residual solution was dried, and glpc analysis indicated the presence of 1.62 g of isobutyl-1-d_1 alcohol. The pure compound (1.2 g) was obtained by two successive glpc purifications on a 2.4-m column of 20% TCEP at 100°. The homogeneous product was distilled, and showed $[\alpha]^{25}D$ 0.49° (neat, $l = 1$). The mass spectrum indicated 80.5% deuterium content. The 100% deuterated optically pure compound should have $[\alpha]^{25}D$ 0.61°. A second fermentation experiment gave a sample containing 70.2% D, $[\alpha]^{25}D$ 0.43° (neat, $l = 1$).

B. Reduction of Isobutyraldehyde with (+)-Diisopinocampheyldeuterioborane.—The apparatus consisted of a 100-ml flask equipped with a magnetic stirring bar, a side arm capped with a rubber septum, and an inlet for dry nitrogen. The flask was flamed in a stream of nitrogen and cooled to 0°. A positive pressure of nitrogen was maintained thereafter.

A solution of deuteriodiborane in tetrahydrofuran (59.1 ml, 23.34 mmol BD_3) was placed in the flask and cooled to 0°. To the stirred solution, (−)-α-pinene (51.3 mmol, 6.95 g) was slowly added from a syringe (20 min), and the stirring was continued for 5 hr (0–3°).

To the white suspension, isobutyraldehyde (23.34 mmol, 1.68 g) was added in the course of 5 min, and the stirring was continued overnight at 0–2°. On addition of water, little hydrogen was produced. The reaction mixture was oxidized by adding 3 *N* NaOH (16 ml) followed by 30% H_2O_2 (8 ml) and by stirring at 40° for 1.5 hr. The tetrahydrofuran layer was separated; the aqueous layer was washed with several portions of ether. The organic extracts were combined, washed once with a little brine and dried, and most of the solvent was removed by distillation through a 30-cm packed column. The residual liquid was distilled through a short Vigreux column, and the fraction at bp 70–110° (750 mm) was collected. The product was twice purified by glpc on the TCEP column and then distilled to furnish (1*S*)-isobutyl-1-d_1 alcohol, $[\alpha]^{25}D$ 0.168 ± 0.02° The mass spectrum indicated that the sample contained 100% $(CH_3)_2 \cdot CH \cdot CDHOH$. Assuming that pure (1*S*)-isobutyl-1-d_1 alcohol has $[\alpha]^{25}D$ 0.61° (neat), this product has an optical purity of 27.6%.

The alcohol was converted into the acid phthalate, which was recrystallized from ligroin (90–120°), mp 63–64° (lit.[16] 62.5–65° for the racemate). A 20% ether solution in a 1-dm tube did not show measurable rotation. The nmr spectrum indicated 99.5–100% deuterium content. The regenerated alcohol, obtained by lithium aluminum hydride reduction of the acid phthalate (see below), had the same optical rotation as the starting material.

(−)-(1*R*)-Isobutyl-1-d_1 Alcohol.—The same apparatus as above was employed. A stirred suspension of sodium borodeuteride (37.5 mmol, 1.58 g) in dry tetrahydrofuran (40 ml) was cooled to 0° and freshly distilled boron trifluoride ethereate (50 mmol, 6.3 ml) was added during 15 min. After stirring for 1 hr, (+)-α-pinene (120 mmol, 16.32 g) was added (20 min), and the mixture was stirred overnight at 0–3°. To the white suspension of the reagent, isobutyraldehyde (50 mmol, 3.6 g) was added during 20 min, and stirring was continued for 4 hr at 0–2°. Addition of water did not generate hydrogen, suggesting that the reduction was complete. The organoborane was oxidized ($NaOH$–H_2O_2), and the product was isolated in the manner described for the (+) enantiomer. The fraction at bp 90–110° (750 mm) was converted into the acid phthalate (90% yield). The acid phthalate was dissolved in a sodium bicarbonate solution and recovered after acidification. The purified product was twice crystallized from ligroin (90–120°), mp 62–63°. A 37% solution of the phthalate in ether in a 1-dm tube did not show a measurable optical rotation.

To the purified acid phthalate (8.0 g) in ether (75 ml), lithium aluminum hydride (2.5 g) was added in small amounts and the mixture was refluxed for 2 hr. The reaction was terminated with dilute hydrochloric acid until an easily filterable white precipitate was obtained. The ether layer was isolated by decantation, the precipitate was washed with ether, and the combined ether solutions were evaporated through a 30-cm packed column. Short-path distillation of the residue gave 2.2 g (81% yield) of (1*R*)-isobutyl-1-d_1 alcohol, bp 105–106° (750 mm) [lit.[16] 106–108° (748 mm)], $[\alpha]^{25}D$ −0.165 ± 0.02°. The sample was homogeneous when analyzed by glpc on a 2.4-m column of 5% TCEP at 115°. The mass spectrum indicated 100% $(CH_3)_2 \cdot CHDOH$. Hence the sample is 27.1% optically pure.

(+)-Isobutyl-1-d_1 Alcohol.—The racemic alcohol was prepared by the reduction of isobutyraldehyde with lithium aluminum deuteride and was purified by distillation. The mass spectrum indicated 100% $(CH_3)_2 \cdot CH \cdot CDHOH$.

Isobutenyl Acetate.—A mixture of isobutyraldehyde (28.8 g, 400 mmol), acetic anhydride (50.5 g, 500 mmol), and *p*-toluenesulfonic acid hydrate (100 mg) was refluxed for 18 hr. Sodium acetate (2.0 g) and water (50 ml) was added and the solution was stirred for 18 hr. The layers were separated and the aqueous layer was extracted with ether. The extract and the main organic layer were combined and washed with a cold dilute sodium bicarbonate solution and water and dried. The solvent was removed through a 60-cm packed column. The residual material was distilled to furnish isobutenyl acetate (85%): bp 123–125° (760 mm) (lit.[17] bp 124–126°, 124°); ν_{max}^{film} 1750 (strong, –OAc), 1685 cm^{-1} (medium, C=C).

Isobutyraldehyde-2-*d*.—A mixture of isobutenyl acetate (7.5 g), deuterium oxide (5.0 g), and concentrated sulfuric acid (2 drops) was stirred and distilled (water bath) through a Vigreux column during 6 hr. The bulk of the D_2O was removed from the distillate by freezing, anhydrous potassium acetate (200 mg) was added, and the isobutyraldehyde-2-*d* was distilled: bp 62–64° (760 mm); ν_{max}^{film} 1730 cm^{-1}; yield 3.3 g.

Isobutyl-2-*d* Alcohol.—To a solution of isobutyraldehyde-2-*d* (150 mg) and isobutyraldehyde (850 mg) in ether (50 ml), lithium aluminum hydride (500 mg) was added, and the mixture was refluxed for 1 hr. The recovered alcohol was purified by glpc on a 2.5-m column of 20% TCEP on Chromosorb, and by distillation. The homogeneous alcohol contained 17.8% D.

The sample was further diluted with isobutyl alcohol to give a specimen of $(CH_3)_2CD \cdot CH_2OH$ containing 10.9% D which was treated with NAD^+–YADH (see Table I).

Degradation of Methyl (+)-(3*R*)-Isocaproate-3-d_1 (4a) to (1*S*)-Isobutyl-1-d_1 Alcohol (9). A. Diphenylalkene 6.—The ester **4a** (25 mmol, 3.25 g) was added with cooling to phenylmagnesium bromide (60 mmol) in ether (60 ml). The reaction mixture was stirred for 2 hr at room temperature and refluxed for 30 min. Excess dilute hydrochloric acid was added, the ether was separated, and the aqueous phase was extracted twice with ether (25 ml). The combined ether solution was washed with water and dried, and the solvent was removed *in vacuo*. The crude liquid alkyldiphenylcarbinol was dissolved in benzene (150 ml) containing a few crystals of *p*-toluenesulfonic acid. The mixture was slowly distilled, and, when the dehydration was complete (no -OH band in the ir) (2 hr), the reaction was terminated. The solution was washed with aqueous sodium carbonate and water, and evaporated *in vacuo*. The remaining liquid was distilled and furnished the diphenylalkene **6** (4.4 g, 75% yield): bp 115–116° (0.2 mm); no hydroxyl absorption in ir; mass spectrum 237 (M^+), 194 (M − 43), (M − 83), etc. Analysis by glpc at 225° on a 2.4-m column of 5% SE-30 on Chromosorb showed that the sample was contaminated with a small amount of bromobenzene and traces of unidentified impurities. This material was employed in the next step.

B. (2*S*)-Isovaleraldehyde-2-d_1 (7).—A solution of diphenylalkene **6** (2.6 g) in methylene chloride (20 ml) was cooled in Dry Ice–Methyl Cellosolve and ozonized for 2.5 hr until the blue color persisted. The solution was then stirred for 4 hr with zinc dust (2.5 g) and glacial acetic acid (2.0 ml) at room temperature. The excess acid was removed by stirring the mixture with sodium bicarbonate (3 g) and a few drops of water for 1 hr. Then anhydrous sodium sulfate was added and stirring was continued for 30 min. The mixture was filtered into a receiver and cooled in ice, and the solid was washed with several small portions of methylene chloride. Most of the methylene chloride was removed by distillation through a 30-cm packed column. The column was removed, the flask was immersed in an oil bath maintained at 160°, and the distillate (*ca.* 25 ml) was collected in a receiver cooled in ice. The distillation flask was cooled to room temperature, 3 ml of methylene chloride was added, and the mixture was again distilled (160°) almost to dryness. The operation was repeated three times. The distillate, *ca.* 40 ml, contained 593 mg (64%) of isovalerylaldehyde-2-d_1 (7) by glpc.

(16) E. L. Eliel and D. W. Delmonte, *J. Amer. Chem. Soc.*, **80**, 1744 (1958).

(17) P. Z. Bedoukian, *ibid.*, **66**, 1325 (1944).

TABLE I

DEUTERIUM CONTENT AND OPTICAL PURITY OF COMPOUNDS

Entry no.	Compd	Origin	Average %D from MS	% optical purity
1	(+)-(3*R*)-Isocaproic-3-d_1 acid[a]	(+)-(3*R*)-4-Methyl-3-d_1-pentan-1-ol	100	34.0
2	(−)-(3*S*)-Isocaproic-3-d_1 acid[a]	(−)-(3*S*)-4-Methyl-3-d_1-pentan-1-ol	100	32.6
3	(1*S*)-Isobutyl-1-d_1 alcohol	Entry 1	100	34.0
4	(1*R*)-Isobutyl-1-d_1 alcohol	Entry 2	100[h]	32.6
5	Isobutyraldehyde-1-*d*	Entry 3–NAD$^+$, YADH[b]	84.9[c]	...
6	Isobutyraldehyde-1-*d*	Entry 4–NAD$^+$, YADH	57.8[c]	...
7	(+)-(1*S*)-Isobutyl-1-d_1 alcohol	Ald[d]–(+)-reagent[e]	100	27.6
8	(−)-(1*R*)-Isobutyl-1-d_1 alcohol	Ald[d]–(−)-reagent[e]	100	27.1
9	Isobutyraldehyde-1-*d*	Isobutyronitrile–LTEAH[f]	100[c]	...
10	Isobutyraldehyde-1-*d*	Isobutyl-1-d_2 alcohol–LTA[j]	98.4[c]	...
11	(+)-(1*S*)-Isobutyl-1-d_1 alcohol	Entry 9–yeast	80.5	100[i]
12	(+)-(1*S*)-Isobutyl-1-d_1 alcohol	Entry 10–yeast	70.2	100[i]
13	(±)-Isobutyl-1-d_1 alcohol	Ald[d]–LAD[g]	100	...
14	Isobutyraldehyde-1-*d*	Entry 7–NAD$^+$, YADH	77.8[c]	...
15	Isobutyraldehyde-1-*d*	Entry 8–NAD$^+$, YADH	55.5[c]	...
16	Isobutyraldehyde-1-*d*	Entry 11–NAD$^+$, YADH	78.7[c]	...
17	Isobutyraldehyde-1-*d*	Entry 12–NAD$^+$, YADH	71.2[c]	...
18	Isobutyraldehyde-1-*d*	Entry 13–NAD$^+$, YADH	68.0[c]	...
19	Isobutyl-2-*d* alcohol[k]		10.9	...
20	Isobutyraldehyde-2-*d*	Entry 19–NAD$^+$, YADH	8.1[c]	...

[a] Reference 12. [b] NAD$^+$, nicotinamide adenine dinucleotide; YADH, yeast alcohol dehydrogenase. [c] Of the 2,4-dinitrophenylhydrazone. [d] Ald, isobutyraldehyde. [e] Reagent, diisopinocamphenyldeuterioborane. [f] Lithium triethoxyaluminohydride [H. C. Brown and C. P. Garg, *J. Amer. Chem. Soc.*, **86**, 1085 (1964)]. [g] Lithium aluminum deuteride. [h] Of the 3,5-dinitrobenzoate. [i] Assumed, corrected for 100% deuterium. [j] By oxidation of isobutyl-1-d_2 alcohol with lead tetraacetate in pyridine [R. E. Partch, *Tetrahedron Lett.*, 3071 (1964)]. The isobutyl-1-d_2 alcohol was prepared by the reduction of neooctylisobutyrate with lithium aluminum deuteride in ether. [k] See Experimental Section.

The 2,4-dinitrophenylhydrazone derivative had mp 118–121° (lit.[15] for nondeuterated derivative, 123°).

C. (1*S*)-Isobutyl-1-d_1 Alcohol (9).—The above dried solution of isovaleraldehyde-2-d_1 (**7**)(560 mg) in methylene chloride (40 ml) was mixed with freshly dried sodium hydrogen phosphate (5.0 g) and stirred at 0°. A solution of trifluoroperacetic acid (prepared by mixing 2.7 g of trifluoroacetic anhydride and 540 mg of 80% hydrogen peroxide in 10 ml of methylene chloride at 0°) was added during 15 min, and stirred overnight at 0–3°. The mixture was filtered into a cooled flask, and the solid was washed with small amounts of methylene chloride. The filtrate was stirred with sodium bicarbonate (3.0 g) and a few drops of water. After 1 hr, anhydrous sodium sulfate was added, and then filtered into a cooled flask. The solid was washed with small amounts of methylene chloride, and the washings were combined with the main filtrate. The solution was concentrated by distilling through a 30-cm packed column. Analysis (glpc, 20% TCEP column) of the concentrated solution indicated the presence of isobutyl-1-d_1 alcohol (10%), the formate of **8** (45%), a small amount of isovaleraldehyde-2-d_1, and methylene chloride. The formate and the isobutyl-1-d_1 alcohol were isolated by preparative glc (20% TCEP column).

To a solution of the formate in ether, lithium aluminum hydride was added and the mixture was stirred for 30 min at ambient temperature. The reaction was terminated by addition of a few drops of water, and the resultant solid was separated by filtration. The solution was added to the alcohol obtained from glc, and the solvent was removed by distillation through a packed colum (30 cm). The residue was distilled to yield (1*S*)-isobutyl-1-d_1 alcohol (120 mg). The product was contaminated with a small amount of diethyl ether (glpc). The mass spectrum indicated the presence of 100% D.

Degradation of Methyl (−)-(3*S*)-Isocaproate-3-d_1 (5a) to (1*R*)-Isobutyl-1-d_1 Alcohol (12).—The degradation was carried out as described for the (+)-(3*R*)-3-d_1 ester. The derived (2*R*)-isovaleraldehyde-2-d_1 (**10**) was submitted to a Baeyer–Villiger oxidation, and, after $LiAlH_4$ reduction of the formate **11**, (1*R*)-isobutyl-1-d_1 alcohol (**12**) was obtained. The mass spectrum showed 100% d_1; the 3,5-dinitrobenzoate derivative had mp 84–86° (lit.[15] 87° for nondeuterated alcohol); the mass spectrum of the 3,5-dinitrobenzoate derivative showed 99.5–100% D.

General Procedure for the Oxidation of Isobutyl-1-d_1 Alcohols with NAD$^+$ and Yeast ADH.—Preliminary experiments demonstrated that the rate of oxidation of isobutyl alcohol or isobutyl-1-d_1 alcohol was slow. The reagents appeared to decompose and discolor rapidly at 37° at pH 9.5. The decomposition was considerably slower when the oxidation was carried out at 24–25°.

The following procedure was found to be reproducible and was therefore used throughout. The oxidation medium was prepared by dissolving NAD$^+$ (253 μmol, 181.3 mg of an 87% pure sample), and yeast ADH (13 mg, 80% pure) in a 0.25 *M* glycine–sodium hydroxide buffer of pH 9.8 (65 ml). A 2-ml aliquot was removed, diluted with the buffer to 10 ml, and used as a blank for uv. The isobutyl-1-d_1 alcohol (253 μmol, 18.7 mg) was added to the medium, and the oxidation was followed by measuring the optical density at 340 mμ of aliquots five times diluted with the buffer. The amount of NADH formed was calculated[18] on the basis of ϵ 6220. The amount of aldehyde produced was assumed to be equivalent to the amount of NADH.

Normally, the oxidation came to an equilibrium after 17–20 hr, at which time 35–40% of the alcohol was oxidized. In some instances it was found advantageous to add more NAD$^+$ in order to expedite the reaction. The reaction was terminated with a solution of 2,4-dinitrophenylhydrazine (200 mg) in 10% sulfuric acid (25 ml). The flask was warmed briefly at 40–50° and stored in a refrigerator for 2–3 hr. The solids were isolated by centrifugation and washed with water. The solid was mixed with anhydrous sodium sulfate and digested several times with warm methylene chloride. The extracts were combined and evaporated. The resulting residue was twice chromatographed [tlc, silica gel, benzene–hexane (4:1)], the zone corresponding to authentic material was collected, and the product was recovered. All experiments were carried out at least in duplicate, and the average results are given in Table I.

Registry No.—Isobutyraldehyde-1-*d*, 20440-12-4; **9**, 20446-26-8; **12**, 20446-27-9; (±)-isobutyl-1-d_1 alcohol, 20446-28-0; isobutyl-2-*d* alcohol, 20440-13-5; isobutyraldehyde-2-*d*, 4303-51-9.

(18) B. L. Horecker and A. Kornberg, *J. Biol. Chem.*, **175**, 385 (1948).