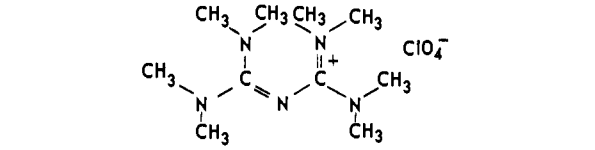
More informative are the nmr spectra of 2-methoxy-1,1,3,3-tetramethylguanidine (**2b**, bp 55–56° (19 mm) and 2-methoxy-1,1,2,3,3-pentamethylguanidine perchlorate (**3b**, mp 181°), the reaction products of tetramethylchloroformamidine chloride and methoxyamine and O,N-dimethylhydroxylamine, respectively. As in the case of **2a**, the spectrum ($CDCl_3$, 33°) of **2b** consists of three singlets at δ 3.65, 2.80, and 2.65 with a relative intensity of 1:2:2. Here the "*syn–anti*" relationships



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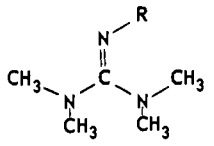
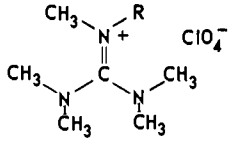


2 a, R = CH_3
b, R = OCH_3



3 a, R = CH_3
b, R = OCH_3

of the $N(CH_3)_2$ groups to the imine methoxyl, a result of restricted rotation about the C=N bond, cause nonequivalence of the methyl groups. The nmr spectrum (D_2O, 33°) of **3b** exhibits singlets at δ 3.67, 3.23, and 2.95 with a relative intensity of 1:1:4. Now the four $N(CH_3)_2$ methyls have become equivalent, and restricted rotation is no longer observed.[7]

The nmr spectra (*o*-$Cl_2C_6H_4$) of **2a** and **2b** display the anticipated temperature-dependent coalescence of the $N(CH_3)_2$ signals. Thus, for **2b** (Figure 1) a coalescence temperature of 152° is observed; this, when substituted into the Eyring equation,[8] permits an estimate of 22.1 kcal/mol for the free energy of activation for rotation. A similar determination for **2a** provides a coalescence temperature of 78°, which corresponds to an energy barrier of 18.8 kcal/mol.

From these observations two conclusions may be drawn. First, in guanidine bases electron localization imparts enough double bond character to the C=N unit to establish restricted rotation. Secondly, stabilization of a guanidinium cation by charge delocalization distributes the double bond character more uniformly to a point where no bond possesses sufficient rigidity to impede rotation relative to the nmr time scale. The great base strength of guanidines is generally considered to be a reflection of the high resonance stabilization of the planar cation.[9] It is thus paradoxical that charge delocalization lowers the energy level of the planar cation and at the same time increases the rate at which rotation from the planar conformation occurs.

From these observations, an explanation for the simple nmr spectrum of **1** may be extrapolated. Charge delocalization over the five nitrogen atoms of **1** results in a distribution of the multiple bond orders so that rotation is not restricted, and complete methyl equivalence is therefore observed.

(7) The possibility that accidental equivalence can account for this observation is remote, particularly since the same phenomenon has been observed in a number of related compounds (to be published).

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Direct Formylation of a Methyl Group in the "Oxo" Reaction. The Formation of (*R*)-3-Ethylhexanal in the Hydroformylation of (+)-(*S*)-3-Methyl-1-hexene

Sir:

In the hydroformylation of (+)-(*S*)-3-methyl-1-pentene, 4% of 3-ethylpentanal is obtained even under reaction conditions wherein practically no double bond migration occurs.[1] In order to clarify the mechanism of formation of this aldehyde, the products of hydroformylation of (+)-(*S*)-3-methyl-1-hexene (I) under similar conditions were examined.

I, optical purity 9.8% (see Table I), was prepared from the racemic compound using *cis*-dichloro-[(*S*)-α-phenylethylamine](ethylene)platinum as previously described.[2]

The hydroformylation of I (35 g, 0.357 mol) was carried out in the presence of $Co_2(CO)_8$ (0.300 g) at 110°, p_{CO} 100 atm and p_{H_2} 100 atm using benzene (40 g) as solvent.

The reaction mixture containing three major reaction products in the ratio 93.0:3.4:3.6 was oxidized with silver oxide and the mixture of acids thus obtained was esterified by an excess of CH_2N_2.

The mixture of the esters (41.6 g, 0.285 mol; ratio between the three major products, corresponding to about 99% of the total products, 93.8:3.1:3.1 (Perkin-Elmer vapor fractometer F 11, polypropylene glycol on Chromosorb W (15:85), 4 ft, 120°)) was separated by vapor phase chromatography (Perkin-Elmer fractometer F 21, butanediol succinate polyester (LAC-6-R-860) on Chromosorb A (20:80), 10 ft, 140°).

Three fractions were obtained which were shown to be respectively methyl (+)-(*S*)-4-methylheptanoate (II) (35 g), a mixture of diastereoisomeric methyl 2,3-dimethylhexanoates (III) (0.700 g), and methyl (+)-(*R*)-3-ethylhexanoate (IV) (0.690 g).

II has been identified by reduction ($LiAlH_4$) as (+)-(*S*)-4-methyl-1-heptanol,[3] bp 95° (20 mm), $n^{22}D$ 1.4290.

The structure of III was demonstrated by comparing its infrared and nmr spectra with those of an authentic sample of a diastereoisomeric mixture of racemic methyl 2,3-dimethylhexanoates, prepared as previously described.[4]

Finally, IV ($n^{25}D$ 1.4164) has been identified as methyl (+)-(*R*)-3-ethylhexanoate by comparing its infrared and nmr spectra with those of an authentic

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(2) R. Lazzaroni, P. Salvadori, and P. Pino, *Tetrahedron Lett.*, 2507 (1968).

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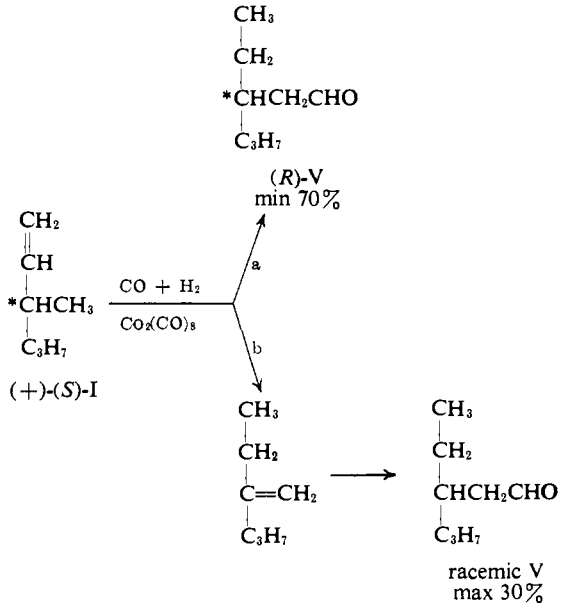
Table I

Compound	$n^{25}D$	α^{25}_{365}[a]	α^{25}_{589}[a]	$[M]^{25}_{589}$	Optical purity, %
(+)-(*S*)-3-Methyl-1-hexene (I)	1.3940	+6.608°	+1.963°	+2.79°	9.8[b]
Methyl (+)-(*S*)-4-methylheptanoate (II)	1.4165	+0.582°	+0.187°	*d*	*d*
Methyl (−)-(3*S*)-2,3-dimethylhexanoate (III)	1.4162	−5.640°	−1.735°	*d*	Diastereoisomeric mixture
Methyl (+)-(*R*)-3-ethylhexanoate (IV)	1.4164	+0.218°	+0.069°	+0.125°	6.8[c]

[a] $l = 1$. [b] Determined by hydrogenation of the olefin to the corresponding paraffin and assuming for optically pure (−)-(*R*)-3-methylhexane $[M]^{25}D$ −9.9° [P. A. Levene and R. E. Marker, *J. Biol. Chem.*, **103**, 302 (1933)]. [c] Calculated by comparison of its optical rotatory power with the one of a sample of antipodic IV of known optical purity. The reference sample of methyl (−)-(*S*)-3-ethylhexanoate, bp 76° (19 mm), $n^{25}D$ 1.4164, $[M]^{25}D$ −0.449° (*Anal.* Calcd for $C_9H_{18}O_2$: C, 68.31; H, 11.47. Found: C, 68.21; H, 11.66), was prepared by treating (−)-(*S*)-3-ethylhexanoic acid ($[M]^{25}D$ −0.870°, optical purity 24%) with an excess of CH_2N_2. In the literature [P. A. Levene, A. Rothen, and C. M. Meyer, *J. Biol. Chem.*, **115**, 401 (1936)], $[M]^{25}D_{max}$ −3.41° is reported for optically pure (−)-(*S*)-3-ethylhexanoic acid. We have obtained $[M]^{25}D$ −3.60° for optically pure (−)-(*S*)-3-ethylhexanoic acid prepared from (+)-(*S*)-1-chloro-2-ethylpentane through carboxylation of its Grignard reagent which by hydrolysis yielded (+)-(*S*)-3-methylhexane having $[M]^{25}D_{max}$ +9.9°. [d] Not determined.

Chart I

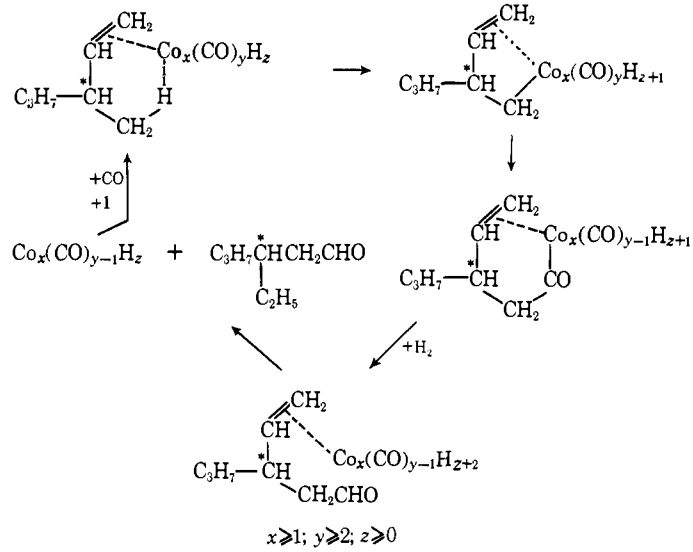


occurring during the formation of (*R*)-3-ethylhexanal (V) is 30%. Therefore, at least 70% of the product is formed through a process involving neither racemization nor inversion but only, in our opinion, through direct formylation of the methyl group in position 3 (Chart I, path a). The remaining 30% might be formed by previous isomerization of I to 2-ethyl-1-pentene which, by hydroformylation, could obviously yield only racemic product (Chart I, path b).

The abstraction of a hydrogen atom initially bound to a saturated carbon atom is not unexpected in the chemistry of the transition metal complexes.[6] Such abstraction has been postulated in the double bond migration catalyzed by $Fe(CO)_5$[7] and it has been demonstrated in the dimerization of butadiene catalyzed by $CoC_{12}H_{19}$.[8] A possible path for the CH_3 formylation is indicated in Chart II.

It is, however, not clear in this formulation what type of bonding exists between the olefinic group and the cobalt carbonyl hydride since this nonconjugated olefinic group apparently is only hydrogenated when the methyl group is formylated.

Chart II



sample obtained by esterification with CH_2N_2 of (−)-(*S*)-3-ethylhexanoic acid prepared according to Levene, *et al.*[5]

By comparing the optical purity of I and IV (see Table I) it appears that the maximum racemization

It is possible that the direct formylation of methyl groups plays an important role in determining the overwhelming formylation of position 1 of internal

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olefins under conditions where the migration of the double bond occurs only to a small extent.[9]

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Enzymatic Displacement of Oxygen and Sulfur from Purines[1]

Sir:

Aminohydrolases have been found to catalyze hydrolytic removal of both nitrogen[2-6] and halogen substituents from the 6 position of purine derivatives. 6-Methoxypurine ribonucleoside was also found to be hydrolyzed to inosine,[3,4] but the position of bond cleavage was not determined. For these reasons, and because adenosine deaminase from takadiastase removes different substituents at similar limiting rates, hydrolysis *via* a purinyl–enzyme intermediate was proposed.[3,4] In contradication to this mechanism, an apparent exchange of radioactive hypoxanthine into 6-chloropurine was observed with bacterial adenine deaminase;[7] this was later found to be entirely due to a radioactive impurity.[8] Indeed the completely negative results obtained by these authors and ourselves[9] render an ammonia–enzyme or chloro–enzyme complex highly improbable.

We wish to report that adenosine deaminases from both takadiastase and calf duodenum, recently isolated as homogeneous proteins,[10] catalyze exchange of oxygen from $H_2{}^{18}O$ into inosine. Inosine is relatively stable,[11] partly due to tautomerization to the keto form,[12] so that exchange is rather slow and was not detected in earlier tests for rapid exchange.[13] Indeed exchange occurs so much more slowly than hydrolysis of methoxypurine ribonucleoside that it has also been possible to determine the position of bond cleavage of 6-methoxypurine ribonucleoside.

Samples containing 80 mg of inosine in 5 ml of 1.40 atom % excess $H_2{}^{18}O$, containing 0.1 *M* sodium acetate buffer, pH 5.4, were incubated at 25° with varying amounts of enzyme (units as described in ref 14) for 24 or 48 hr, along with controls containing no enzyme and controls containing adenosine and enzyme to provide fully labeled inosine after hydrolysis. The inosine concentration chosen in these experiments greatly exceeded its dissociation constant from either enzyme, as indicated by its K_i as an inhibitor of adenosine deamination.[14] Reaction was stopped and inosine was converted to hypoxanthine by heating samples for 1 hr at 100° in the presence of 0.3 *M* H_2SO_4. Adjustment to pH 5 with KOH precipitated hypoxanthine, which was washed with cold water, dried *in vacuo*, and converted to carbon dioxide by heating with mercuric chloride at 400° for 2 hr.[15] Excess HCl was removed by passing the pyrolysis products through a 7,8-benzoquinoline column. The oxygen-18 content of the carbon dioxide thus obtained was determined from the relative peak heights of the *m/e* 46 to 44 ratios on a CEC 21-614 residual gas analyzer, modified with an inlet for batch sample analysis.[16] The results, shown in Figure 1 and Table I, correspond to exchange rates lower by approximately five orders of magnitude than the limiting rate constants for adenosine deamination by the calf and takadiastase enzymes.

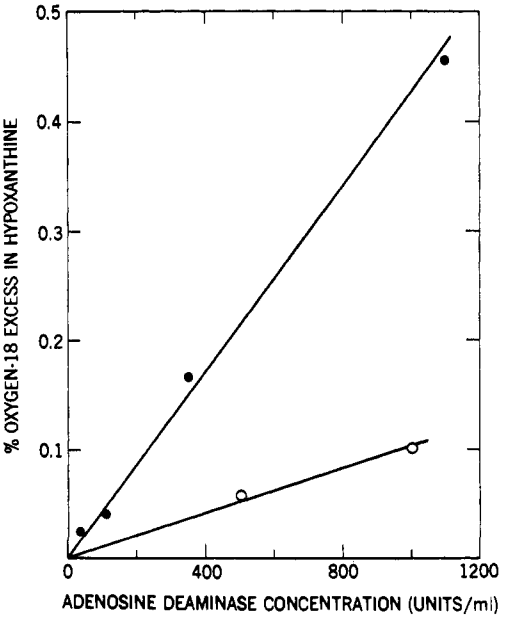

Figure 1. Per cent oxygen-18 in hypoxanthine recovered from inosine which had been incubated 24 hr with the indicated concentration of adenosine deaminase (corrected for nonenzymatic incorporation, 0.02% excess). Conditions: 80 mg of inosine, in 5.0 ml of 0.1 *M* sodium acetate buffer, pH 5.4, at 25°; 1.24% $H_2{}^{18}O$ excess; (●) enzyme prepared from calf intestine; (○) takadiastase enzyme.

In a second set of experiments, 6-methoxypurine ribonucleoside was hydrolyzed in $H_2{}^{18}O$ in the presence of the takadiastase enzyme (125 units/ml) for 4 hr at 25.[3] The resulting inosine was found to be within experimental error, fully labeled with ^{18}O. A parallel control containing inosine and enzyme underwent

(1) The work at Princeton University was supported by Research Grant GM-12725 from the National Institutes of Health, U. S. Public Health Service. The work at the University of California was supported by Research Grants GM-12278 from the National Institutes of Health, U. S. Public Health Service, and GB-4606 from the National Science Foundation.

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