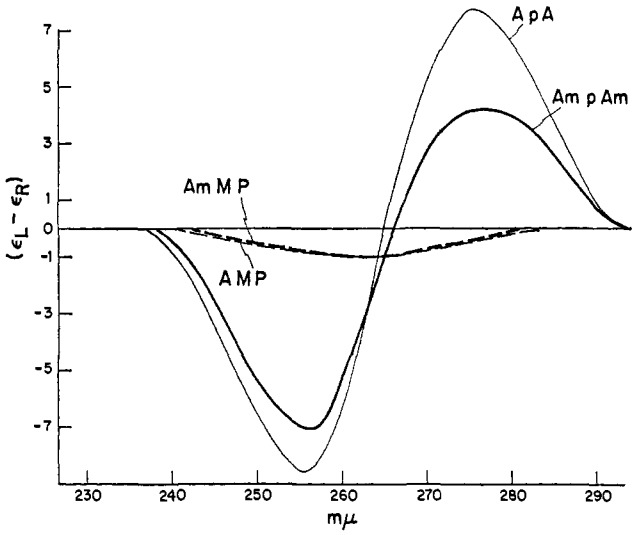

Figure 1. CD spectra of AMP, AmMP, ApA, and AmpAm in 4.7 *M* KF–0.01 *M* Tris buffer, pH 7.5, at 27°. A Cary 60 recording spectropolarimeter equipped with a 6001 CD-accessory and a JASCO Model ORD/UV/CD-5 were used. Differential dichroism absorption is given on a "per nucleotide residue" basis.

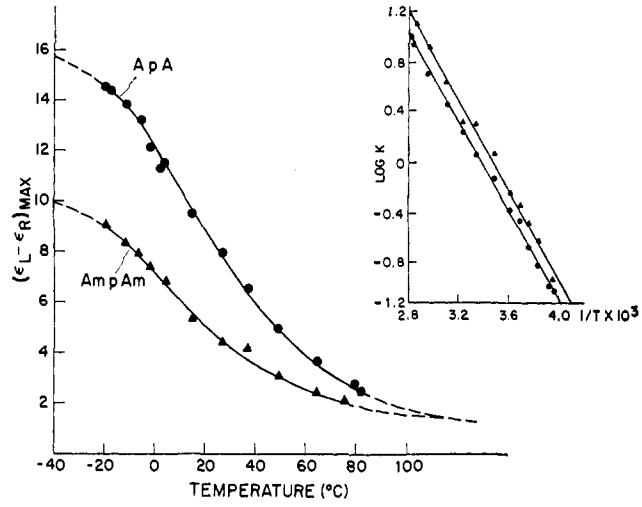

Figure 2. CD–temperature profiles of ApA and AmpAm in 4.7 *M* KF–0.01 *M* Tris buffer, pH 7.5. Differential dichroism absorption at the maximum of the positive band is plotted against temperature. Very similar melting curves were obtained in the region from 5 to 60° in 0.15 *M* NaCl. The inset gives a van't Hoff plot derived from these data on the basis of a two-state model. The high- and the low-temperature ends of the melting curves are approximated.

considerably lower for AmpAm; (2) the spectrum is less conservative; (3) the crossing point is shifted to slightly longer wavelength.[13] The spectral differences indicate that a slight change in the orientation of the bases has occurred in ApA upon 2′-O-methylation which has not been readily discernible from the nmr spectra. CD temperature profiles of the two dinucleotides are shown in Figure 2. Both profiles have sigmoidal shape. A somewhat lower T_m is obtained for AmpAm (approximately 15°) than for ApA (approximately 21°) from the curves. A van't Hoff plot derived from the melting data on the basis of a two-state model is given in the inset of Figure 2. Rather close values are obtained for the thermodynamic parameters $\Delta H°$, $\Delta S°$, and $\Delta F°$ (at 0°) for the methylated and unmethylated dinucleotide.[14]

The following general conclusions can be reached from our results. Although a small change in the three-dimensional structure and the stability of the ordered conformation of ApA does occur upon methylation of the 2′-hydroxyl groups, the change is not as substantial as for the case where the 2′-hydroxyl groups are replaced by hydrogens as in dApdA.[2] It appears, therefore, that an important requirement for the increased stability of oligo- and polyribonucleotides relative to their deoxyribonucleotide counterparts is the presence of an oxygen atom in the 2′ position of the sugar. The hydrogen bond donor capabilities of unsubstituted 2′-hydroxyl groups, however, do not seem to be essential. Based on the results obtained with arabinosyl dinucleotides[15,16] it is most likely that the stereochemistry of carbon 2 of the sugar is furthermore of importance. It is not possible at present to decide if the 2′-oxygen atoms exert their stabilizing effect on RNA conformation due to their properties as hydrogen bond acceptors, van der Waals interactions, or both.

Acknowledgment. We have profited in the course of this work from discussions with Walter Kauzmann. This research was supported by Grant GM-14090 from the National Institutes of Health, by Grant GB-4781 from the National Science Foundation, and by a grant from Hoffman-La Roche Inc., Nutley, N. J.

(13) Since 2′-O-methylation has introduced changes both in the shape and the intensity of the CD bands, the spectral differences between ApA and ApmAm cannot be adequately interpreted on the basis of simple exciton theory.

(14) Identical values are obtained for the enthalpies of the disordering process for both dinucleotides ($\Delta H° = 8.1$ kcal/mol), while $\Delta S°$ is slightly higher for AmpAm (28 eu/mol) than for ApA (27 eu/mol). Correspondingly, $\Delta F°$ at 0° is lower for AmpAm (0.3 kcal/mole) than for ApA (0.6 kcal/mole). The larger value for $\Delta S°$ and the lower stability of the ordered structure of AmpAm may be due to a larger amount of rotational freedom gained in the disordering process of AmpAm than of ApA. Model building (Corey–Pauling–Koltum models) indicates that introduction of the bulky methyl substituents may diminish the flexibility of the backbone predominantly in the ordered conformation of AmpAm. A change in solvation resulting from the replacement of the polar hydroxyls by essentially nonpolar methoxy groups may also be of importance. While this interpretation seems plausible, the following should be kept in mind: for the determination of the T_m's and the derivation of the thermodynamic data the high- and the low-temperature branches of the melting curves have to be approximated. This may give rise to considerable error, especially for the low-temperature ends of the curves. Furthermore, the thermodynamic treatment is based on the two-state model which is not clearly established.

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(16) A. J. Adler, L. Grossman, and G. D. Fasman, *Biochemistry*, **7**, 3836 (1968).

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The Mechanism of α-Ketoglutarate Oxidation in Coupled Enzymatic Oxygenations[1]

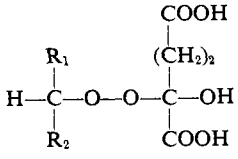
Sir:

A group of hydroxylases requires α-ketoglutarate as a cofactor.[2–6] For γ-butyrobetaine hydroxylase[7] and

(1) This work was supported by grants from the Swedish Medical Research Council (13 X-585) and Alfred Österlunds Stiftelse.

collagen proline hydroxylase[8] it has been shown that 1 mole of α-ketoglutarate is decarboxylated per mole of hydroxylated product and that succinate is formed.[9] From experiments with γ-butyrobetaine hydroxylase we have concluded that free succinic semialdehyde is not an intermediate in this reaction.[10] We therefore suggested a concerted reaction mechanism in which one atom of the oxygen molecule is incorporated into succinic acid, the other into the hydroxylated product.[10]

The anion of an initially formed hydroperoxide of the substrate to be hydroxylated would attack the α carbon of the keto acid, resulting in the formation of an intermediate complex of the type



Model experiments in our laboratory have given support for a reaction mechanism of this type; *e.g.*, *t*-butyl alcohol and succinic acid are formed in the reaction between *t*-butyl hydroperoxide and α-ketoglutarate. To establish that molecular oxygen is incorporated into succinate in the enzyme reaction, as required by the above mechanism, we have carried out the hydroxylation of γ-butyrobetaine in an $^{18}O_2$-enriched atmosphere.

A partially purified preparation of γ-butyrobetaine hydroxylase from *Pseudomonas* AK 1[11,12] was incubated with γ-butyrobetaine (34 μmoles) and α-ketoglutarate (33 μmoles) in a phosphate buffer at pH 7.0 containing Fe^{2+} (7 μmoles) and ascorbate (171 μmoles) in a total volume of 12 ml. The reaction mixture was flushed with argon immediately before addition of the enzyme, quickly frozen in liquid nitrogen, and connected to a manifold from which $^{18}O_2$ gas (90% $^{18}O_2$) was added. At the end of the incubation period (95 min) the reaction mixture was acidified to pH 2–3 with sulfuric acid and immediately extracted five times with three volumes of diethyl ether. The ether was evaporated under vacuum at room temperature and the residue was dissolved in 50 μl of dry pyridine, to which was added 100 μl of bis(trimethylsilyl)trifluoroacetamide to convert succinic acid to the bis(trimethylsilyl) ester. This derivative had the same retention time as the bis(trimethylsilyl) ester of authentic succinic acid when subjected to gas chromatography at 90° on a column of 2% SE-52. The ^{18}O content was determined in an instrument for combined gas chromatography–mass spectrometry at the Department of Medical Biochemistry, University of Gothenburg.[13] Figure 1 shows the mass spectrum obtained, from which it is apparent that the formed succinic acid contained about 75% of molecules with one atom of ^{18}O.[14] According to present nomenclature[15] monooxygenases incorporate one atom of the oxygen molecule into the substrate whereas the other is reduced to water by a reducing cofactor, *e.g.*, NADPH, ascorbate, or a tetrahydropteridine. Dioxygenases on the other hand catalyze the incorporation of both oxygen atoms into the substrate. The enzymes of which γ-butyrobetaine hydroxylase is an example constitute another group of oxygenases for which an α-keto acid is the reducing factor. In the biological systems discussed above there is a high specificity for α-ketoglutarate, whereas in the model system other keto acids may substitute for α-ketoglutarate.[16]

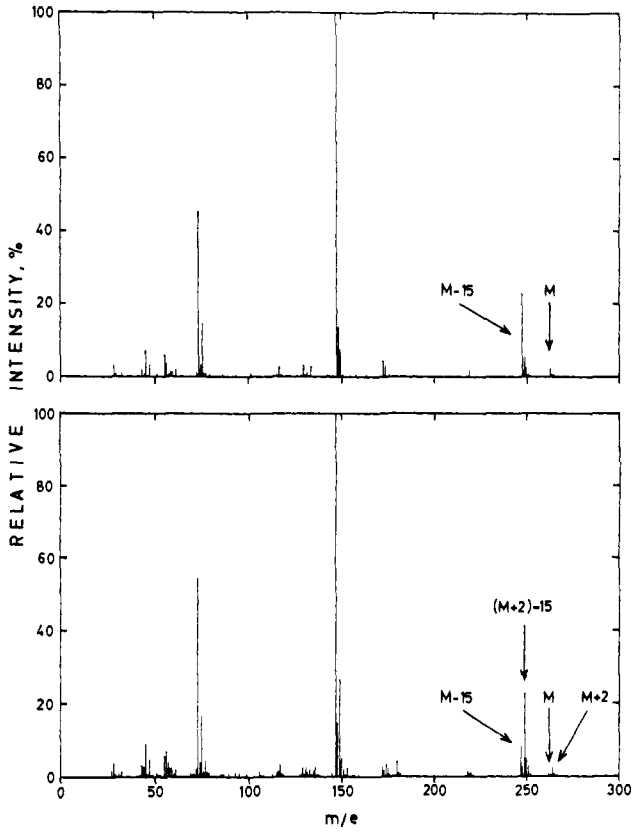


Figure 1. Mass spectrum of the bis(trimethylsilyl) ester of succinic acid formed during the incubation of γ-butyrobetaine and α-ketoglutarate with γ-butyrobetaine hydroxylase in 90% $^{18}O_2$ (*lower graph*) and of bis(trimethylsily) ester of authentic succinic acid (*upper graph*). M is the molecular ion and M − 15 the positive ion after loss of one methyl radical from a trimethylsilyl group of the ester. The incorporation of one atom of ^{18}O in about 75% (see text) of the formed succinic acid is evident from the presence of the corresponding ions plus 2 mass units in the lower graph.

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(11) G. Lindstedt, S. Lindstedt, M. Tofft, and T. Midtwedt, *Biochemistry*, 6, 1262 (1967).
(12) An extract of sonicated cells had been purified by chromatography on DEAE-cellulose and hydroxylapatite. Details of this procedure will be given elsewhere.
(13) We are greatly indebted to Professor Stina Stenhagen for help with the mass spectrometric work.
(14) Incomplete removal of dissolved air and exchange of carboxyl oxygen with water are likely explanations for the deviation from the theoretical 90% ^{18}O.
(15) O. Hayaishi, Proceedings of the Sixth International Congress of Biochemistry, Plenary Sessions, New York, N. Y., 1964, p 31.
(16) After the completion of the present work we incubated *p*-

Interestingly, the reaction opens a pathway for the biological oxidative decarboxylation of α-ketoglutarate other than that catalyzed by α-ketoglutarate dehydrogenase (EC 1.2.4.2) which requires thiamine pyrophosphate, lipoate, and FAD as cofactors.

hydroxyphenylpyruvate hydroxylase (EC 1.14.2.2) in an $^{18}O_2$-enriched atmosphere. Two atoms of molecular oxygen were incorporated into the formed homogentisic acid. Thus, in this case an intramolecular reaction of the same type as the one discussed in this communication had occurred, and *p*-hydroxyphenylpyruvate hydroxylase belongs to the class of oxygenases utilizing α-keto acid as reductant.

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Received May 19, 1969

Reaction of B-Alkyl-9-borabicyclo[3.3.1]nonanes with Carbon Monoxide in the Presence of Lithium Tri-*t*-butoxyaluminohydride. The Conversion of Functionally Substituted Olefins into Aldehydes *via* Hydroboration

Sir:

We wish to report that the B-alkyl-9-borabicyclo-[3.3.1]nonanes[1] (B-R-9-BBN) react readily with carbon monoxide in the presence of lithium tri-*t*-butoxyaluminohydride,[2,3] without reduction of functional substituents. This development makes possible the facile introduction of the aldehyde group into olefins containing many representative functional substituents (eq 1–5).

$$H_2C{=}CH(CH_2)_8CO_2CH_3 \longrightarrow OHC(CH_2)_{10}CO_2CH_3 \quad (1)$$
99%

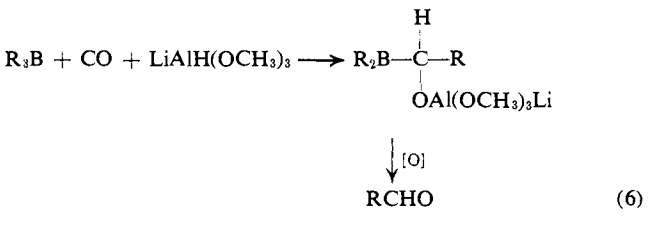
$$H_2C{=}CH(CH_2)_8CN \longrightarrow OHC(CH_2)_{10}CN \quad (2)$$
95%

$$H_2C{=}CHCH_2CO_2C_2H_5 \longrightarrow OHC(CH_2)_3CO_2C_2H_5 \quad (3)$$
83%

$$H_2C{=}CHCH_2CN \longrightarrow OHCCH_2CH_2CH_2CN \quad (4)$$
85%

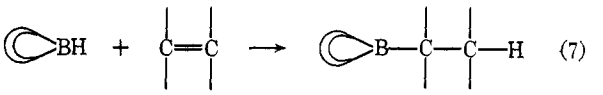
$$H_2C{=}CHCH_2O_2CC_6H_5 \longrightarrow OHCCH_2CH_2CH_2O_2CC_6H_5 \quad (5)$$
89%

We recently reported that carbon monoxide reacts rapidly and essentially quantitatively with trialkylboranes in the presence of lithium trimethoxyaluminohydride, providing a highly useful synthetic route to the corresponding homologated aldehydes[4] (eq 6). Unfortunately, in this synthesis only one of the three alkyl groups in the organoborane is utilized for the production of aldehyde.

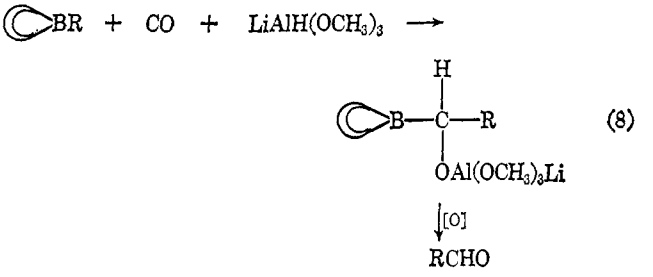


(6)

More recently we noted that the application of the B-alkyl-9-borabicyclo[3.3.1]nonanes (eq 7) overcame this difficulty[5] (eq 8). This development provided an



(7)



(8)

exceptionally simple procedure for introducing an aldehyde group into alkenes and dienes of a wide variety of structures in highly satisfactory yields.[5]

Hydroboration is a mild reaction which can tolerate many functional groups.[6] Consequently, there is no problem in preparing the B-R-9-BBN derivatives containing functional substituents in the B-R group. On the other hand, lithium trimethoxyaluminohydride is a powerful reducing agent,[7] approaching lithium aluminum hydride in effectiveness. It was therefore quite clear that the usual procedure of introducing carbon monoxide into a mixture of the organoborane and metal hydride would be unsatisfactory.

Fortunately, it was observed that the slow addition of a solution of lithium trimethoxyaluminohydride in THF to a well-stirred THF solution of B-R-9-BBN, saturated with carbon monoxide,[8] gave reasonable yields of the desired aldehydes containing reducible functional substituents. Evidently the reagent reacts much more rapidly with the carbonyl intermediate than

Table I. A Comparison of Lithium Trimethoxyaluminohydride and Lithium Tri-*t*-butoxyaluminohydride for the Carbonylation of Functionally Substituted B-Alkyl-9-borabicyclo[3.3.1]nonane Derivatives[a]

Olefin	Product	Yield, %[b] LiAlH-(OCH3)3	Yield, %[b] LiAlH-(O-*t*-Bu)3
Methyl 10-undecenoate	11-Carbomethoxy undecanol	99	99
3-Buten-1-yl acetate	5-Acetoxypentanal	45	92
Allyl benzoate	4-Benzoxybutanal	25	89

[a] All reactions were carried out at −25°, utilizing equimolar amounts of the B-R-9-BBN and the metal hydride. [b] Yields by glpc analysis.

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(3) The reagent is available from the Ventron Corp., Beverly Mass. 01915.
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