Addition Compounds of Alkali Metal Hydrides. 32. A Comparison Study of Chiral Trialkylborohydrides and Chiral Dialkylmonoalkoxyborohydrides for the Asymmetric Reduction of Prochiral Ketones: The Effect of Comparable Chiral Alkyl and Alkoxy Groups on Asymmetric Industion†

Herbert C. Brown*, Won Suh Park[1a], and Byung Tae Cho[1a,b]

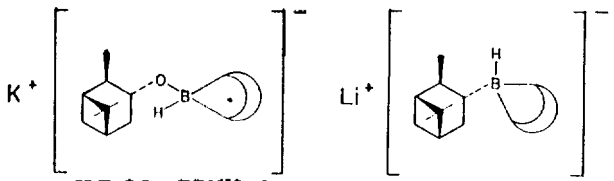
H.C. Brown and R.B. Wetherill Laboratories of Chemistry, Purdue University, West Lafayette, Indiana 47907

Received March 30, 1987

Several chiral potassium B-alkyl-9-boratabicyclo[3.3.1]nonanes (K B-R*-9-BBNH) and potassium *B*-alkoxy-9-boratabicyclo [3.3.1]nonanes (K B-OR*-9-BBNH) were synthesized by treatment of the corresponding trialkylboranes and dialkylmonoalkoxyboranes with a small excess of potassium hydride. The chiral B-alkoxy derivatives generally reduce representative ketones, such as acetophenone and 3-methyl-2-butanone, with greater optical induction than the corresponding B-alkyl derivatives, suggesting the involvement of the oxygen atom in the control process for asymmetric synthesis.

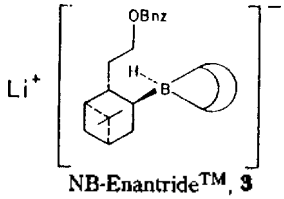
The recent development of synthetic methods for stable dialkylmonoalkoxyborohydrides by using cyclic substituents, such as 9-borabicyclo[3.3.1]nonane (9-BBN), and excess potassium hydride,[2] made possible the development of a number of asymmetric reducing agents belonging to this class.[3] Especially, potassium 9-O-(1,2:5,6-di-O-isopropylidene-α-D-glucofuranosyl)-9-boratabicyclo[3.3.1]nonane, K 9-O-DIPGF-9-BBNH, K-Glucoride, proved to be one of the most promising reagents of this class,[3] affording excellent optical inductions in the reduction of several different classes of ketones.[4] In the hope of achieving an understanding of the major factors controlling the magnitude of the asymmetric inductions achieved in such asymmetric reductions, we undertook to explore the reducing characteristics of such chiral borohydrides with systematic variation in the chiral auxiliary.

In the course of our investigation of the syntheses of various chiral potassium B-alkoxy-9-boratabicyclo[3.3.1] nonanes, K B-OR*-9-BBNH, and their asymmetric reduction of selected ketones,[3b] we encountered an unexpected, but highly interesting result. We noted that the enantioselectivity achieved, 61% ee, in the reduction of 3-methyl-2-butanone with potassium 9-O-isopinocampheyloxy-9-boratabicyclo [3.3.1]nonane, K 9-O-Ipc-9-BBNH, **1**, is considerably better than that achieved, 36% ee, with the corresponding lithium B-isopinocampheyl-9-boratabicyclo[3.3.1]nonane, Li B-Ipc-9-BBNH, **2**,[5] and approaches the value, 68% ee, achieved with the improved reagent, NB-Enantride, **3**.[6]



K B-*O*-Ipc-BBNH, **1**

Li B-Ipc-9-BBNH, **2**



NB-Enantride™, **3**

This observation suggested that the presence of an alkoxy substituent in chiral borohydride reagents may be favorale to achieve high optical yields in such reductions.

Consequently, it appeared desirable to perform a systematic study of the phenomenon by comparing the optical yields realized in the asymmetric reduction of representative ketones with several pairs of chiral trialkylborohydrides and chiral dialkylmonoalkoxyborohydrides.

Thus, in the present paper, we wish to report syntheses of several pairs of K B-OR*-9-BBNH and K B-R*-9-BBNH derived from readily available chiral terpenoid olefins and alcohols. We noted a general improved effect of alkoxy substituents over the corresponding alkyl substituents in providing improved optical yields in the reduction of the test ketones, acetophenone and 3-methyl-2-butanone, with the paired reagents.

Results and Discussion

A variety of stable chiral K B-R*-9-BBNH and K B-OR*-9-BBNH were synthesized by treatment of the corresponding trialkylboranes, B-R*-9-BBN and borinic esters, B-OR*-9-BBN with excess potassium hydride in THF. Those B-R*-9-BBN derivatives, in turn, were prepared by hydroboration of the corresponding terpenoid olefins, such as (+)-α-pinene, (−)-nopolbenzyl ether, (+)-3-carene, (−)-2-carene with 9-BBN. The corresponding B-OR*-9-BBN derivatives were prepared by treating the corresponding terpenoid alcohols, such as (+)-10-benzyloxymethylisopinocampheol and (−)-2-isocaranol, with 9-BBN. The asymmetric reduction of the representative ketones, acetophenone and 3-methyl-2-butanone using these new chiral borohydrides were carried out and the optical yields achieved was established by capillary GC analyses of the MTPA esters of the corresponding product alcohols. The results for K B-OR*-9-BBNH derived from (−)-isopinocampheol and (−)-4-isocaranol were taken from our previous studies.[3b]

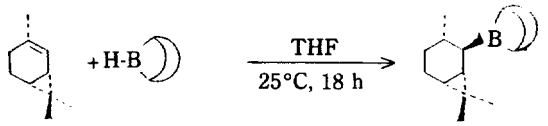
Preparation of Chiral Borohydrides. Chiral B-alkyl-9-BBN derivatives, such as B-Ipc-9-BBN, **4**,[7] NB-Enantrane™, **5**,[6] B-(4-Icr)-9-BBN, **6**,[8] and B-(2-Icr)-9-BBN, **7**, were synthesized by treatment of 9-BBN with the corresponding terpenoid olefins, such as (+)-α-pinene, (−)-nopol

Table 1. Preparation of B-Alkyl-9-BBN and B-Alkoxy-9-BBN in THF[a]

chiral organoborane	temp, °C	time, h	^{11}B NMR, THF δ, ppm (m)
B-Ipc-9-BBN, **4**[b]	65	5.0	82.5(*br s*)
B-O-Ipc-9-BBN[c]	25	2.0	55.5(*br s*)
NB-Enantrane™, **5**[d]	65	18.0	82.9(*br s*)
NB-O-Enantrane, **8**	25	2.0	56.3(*br s*)
B-(4-Icr)-9-BBN, **6**[e]	65	5.0	88.0(*br s*)
B-O-(4-Icr)-9-BBN[c]	25	2.0	55.1(*br s*)
B-(2-Icr)-9-BBN, **7**	25	18.0	84.8(*br s*)
B-O-(2-Icr)-9-BBN, **9**	25	2.0	56.3(*br s*)

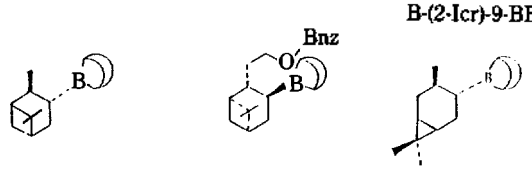
[a]By the reaction of 9-BBN with the corresponding olefins or alcohols. [b]Following the procedure in ref. 7. [c]From ref. 3b. [d]Following the procedure in ref. 6. [e]Following the procedure in ref. 8.

benzyl ether, (+)-3-carene, and (+)-2-carene. The hydroboration reactions for the preparation of **4-6** were carried out following literature procedures. The hydroboration of (+)-2-carene for the preparation of **7** proceeded smoothly at 25°C in THF (eq 1).



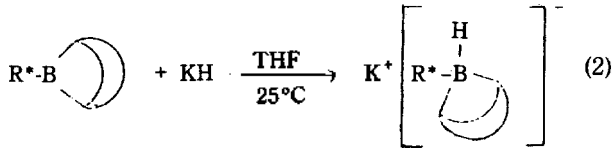
(1)

B-(2-Icr)-9-BBN, **7**

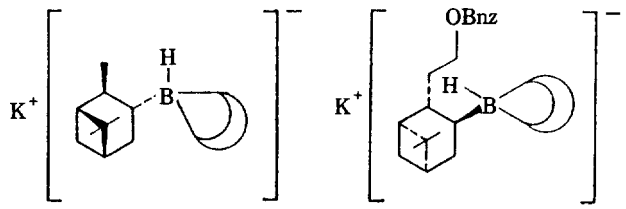


B-Ipc-9-BBN, **4** NB-Enantrane™, **5** B-(4-Icr)-9-BBN, **6**

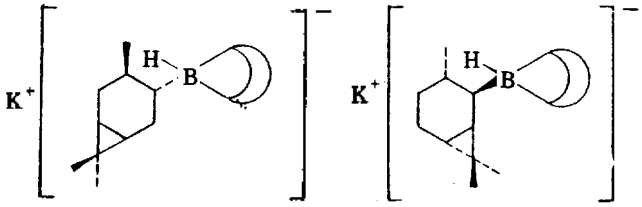
The ^{11}B NMR spectra for the solution in THF revealed complete disappearance of 9-BBN (δ 28.0) with the appearance of only the desired B-alkyl-9-BBN derivatives (δ 82.5-88.0). The reaction conditions and ^{11}B NMR data are summarized in Table 1. The resulting solution was directly utilized for the preparation of the corresponding K B-R*-9-BBNH by treatment with a vigorously stirred suspension of potassium hydride in THF (eq 2).



(2)



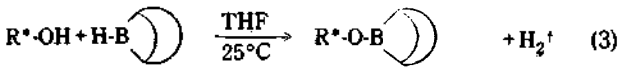
K B-Ipc-9-BBNH, **4**′ K NB-Enantride, **5**′



K B-(4-Icr)-9-BBNH, **6**′ KB-(2-Icr)-9-BBNH, **7**′

The complete disappearance of B-alkyl-9-BBN derivatives and the appearance of characteristic doublet signals were distinct in the ^{11}B NMR spectra with characteristic chemical shifts (δ-6.1-10.3) and coupling constants (70.2-72.1 Hz).

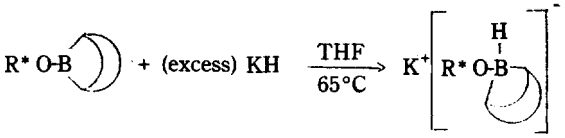
Similarly, chiral B-alkoxy-9-BBN derivatives, such as NB-O-Enantrane, **8**, and B-O-(2-Icr)-9-BBN, **9**, were synthesized by treatment of 9-BBN with the corresponding chiral alcohols, such as (+)-10-benzyloxymethylisopinocampheol and (−)-2-isocaranol following the previously published procedure[3b] (eq 3).



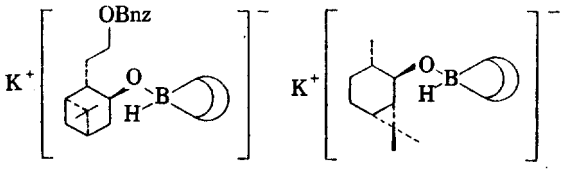
(3)

NB-*O*-Enantrane, **8** B-*O*-(2-Icr)-9-BBN, **9**

The progress of the reaction was conveniently monitored both by ^{11}B NMR and measurement of hydrogen evolution (1 equiv). Both borinic esters exhibit ^{11}B NMR signals at δ 56.3. The results are summarized in Table I. Again, the resulting solutions of the borinic esters were utilized directly for the preparation of the corresponding K B-OR*-9-BBNH, **8**′ and **9**′, by treatment with excess potassium hydride (eq 4). The formation of chiral borohydride can also be conveniently followed by ^{11}B NMR, monitoring and characteristic broad singlet signals at δ-1.8 to 0.6.



(4)



K NB-*O*-Enantride, **8**′ K B-*O*-(2-Icr)-9-BBNH, **9**′

The hydridation of chiral borinic esters, **8** and **9**, requires higher temperature and longer reaction time than that of the trialkylborane derivatives, **4,5,6** and **7**. All the chiral borohydrides thus prepared exhibit characteristic B-H stretching absorption around 2000 cm^{-1} in the IR spectra. All these borohydrides proved stable over an extended period of time

Table 2. Formation and Physical Properties of Chiral Borohydrides[a]

chiral borohydrides	formation temp. °C	formation time, h	^{11}B NMR, THF δ, ppm (J_{B-H}, Hz)	IR, ν_{B-H} cm^{-1}
K B-Ipc-9-BBNH, **4**′	25	1.0	−6.1 (*d*, 72.1)	2000
K B-O-Ipc-9-BNH,[b] **1**	25	2.0	−1.51 (*d*, 75.6)	2003
K NB-Enantride, **5**′	25	12	−6.2 (*d*, 70.2)	2005
K NB-O-Enantride, **8**′	65	48	0.55 (*br s*)	2005
K B-(4-Icr)-9-BBNH, **6**′	25	1.0	−10.3 (*d*, 71.1)	2006
K B-O-(4-Icr)-9-BBNH,[b] **10**	25	2.0	−1.7 (*d*, 85.0)	2006
K B-(2-Icr)-9-BBNH, **7**′	25	1.0	−10.0 (*d*, 71.4)	2004
K B-O-(2-Icr)-9-BBNH, **9**′	65	60	−1.81 (*br s*)	2004

[a]By the reaction of excess potassium hydride with the corresponding chiral organoborane compounds in THF. [b]From ref. 3b.

Table 3. Asymmetric Reduction of Representative Ketones with New Chiral Borohydrides[a]

chiral borohydrides	% ee of alcohol products (config.)[b] acetophenone	3-methyl-2-butanone
K B-Ipc-9-BBNH, **4**′[c]	5[*R*]	41[*R*]
K B-O-Ipc-9-BBNH, **1**[d]	47[*S*]	61[*S*]
K NB-Enantride, **5**′	15[*S*]	32[*S*]
K NB-O-Enantride, **8**′	32[*R*]	62[*R*]
K B-(4-Icr)-9-BBNH, **6**′	7[*S*]	32[*S*]
K B-O-(4-Icr)-9-BBNH, **10**[d]	34[*R*]	28[*S*]
K B-(2-Icr)-9-BBNH, **7**′	3[*S*]	56[*R*]
K B-O-(2-Icr)-9-BBNH, **9**′	49[*R*]	69[*R*]

[a]Reactions in THF at −78°C, [H^-]:[ketone] = 1.1:1.0, [ketone] ≅ 0.3 *M*. [b]Determined by capillary GC analysis of the corresponding MTPA esters. [c]At −50°C. The reagent solidifies at −78°C in THF. [d]From ref. 3b.

when stored under positive pressure of nitrogen in the presence of excess potassium hydride. The stability was monitored both by the ^{11}B NMR spectra and by measuring the number of moles of H_2 evolved in hydrolysis at appropriate time intervals of clear supernatant aliquots. The results on the formation and physical properties of those chiral borohydrides are summarized in Table 2.

Asymmetric Reduction of Prochiral Ketones. In order to study the comparative effects of alkyl *versus* alkoxy substituents in chiral borohydrides on the asymmetric induction, two representative prochiral ketones, acetophenone, an aralkyl ketone, and 3-methyl-2-butanone, an aliphatic ketone, were selected and asymmetric reduction with new chiral borohydrides, **4**′, **5**′, **6**′, **7**′, **8**′ and **9**′ were carried out. Among these new chiral borohydrides, **4**′, **5**′, **6**′ and **8**′ reduce acetophenone and 3-methyl-2-butanone smoothly in THF at −78°C with >90% yields within 24 h (**4**′, at −50°C). However, derivatives **7**′ and **9**′ reduce these ketones more slowly, requiring approximately 72 h to provide >80% yields at the same conditions. The optical yields realized are summarized in Table 3, and compared with the results for two reagents previously studied K B-O-Ipc-9-BBNH, **1**,[3b] and K B-O-(4-Icr)-9-BBNH, **10**.[3b]

Thus, all K B-OR*-9-BBNH derivatives, **1**, **8**′, **10** and **9**′, exhibit significantly better optical yields than the corresponding K B-R*-9-BBNH derivatives, **4**′, **5**′, **6**′ and **7**′ for the asymmetric reduction of acetophenone: 47% *vs* 5% ee, 32% *vs* 15% ee, 34% *vs* 7% ee, 49% *vs* 3% ee respectively. Most of the B-alkoxy derivatives (**1**, **8**′ and **9**′) also reveal better optical inductions than the B-alkyl derivatives (**4**′, **5**′ and **7**′) for 3-methyl-2-butanone: 61% *vs* 41% ee, 62% *vs* 32% ee and 69% *vs* 56% ee respectively. Only one pair, **6**′ and **10**, exhibits the opposite trend. It should be noted that the direction of the change of asymmetric induction in the reduction of 3-methyl-2-butanone with the pair **6**′ and **10** from 32% ee *S* to 28% ee *S* is actually comparable to that observed for the reduction of actophenone with the same pair **6**′ and **10** from 7% ee *S* to 34% ee *R*. In other words, more induction is achieved to the *R*-configuration side of the product by the B-alkoxy derivative (*i.e.*, less to the *S*-configuration side). The best optical yields are achieved by K B-O-(2-Icr)-9-BBNH in the reduction of both of acetophenone and 3-methyl-2-butanone (49% and 69% ee) approaching or slightly better than those achieved by lithium NB-Enantride[6] (70% and 68% ee respectively). It should be noted that the results achieved by the lithium NB-Enantride are considerably better than those achieved by the potassium derivative of the present study (15% and 32% ee respectively). This suggests that the synthesis of the lithium analogues of **9**′ might supply a superior reagent.

The present results indicate that the presence of an oxygen atom in the chiral borohydrides plays an important role in directing the asymmetric incudtion, possibly through the coordinative interactions of the oxygens in the reagent and carbonyl compounds with the positively charged cation in the favorable transition state.

Conclusion

Several pairs of chiral borohydrides belonging to the classes of K B-OR*-9-BBNH and K-B-R*-9-BBNH can be readily synthesized from the corresponding terpenoid alcohols and olefins respectively. When applied to asymmetric reduction of representative ketones, the K B-OR*-9-BBNH derivatives generally exhibit better optical induction than the K B-R*-9-BBNH derivatives, revealing the importance of the oxygen substituent of the alkoxyl groups in favoring asymmetric induction.

Experimental

Materials and General Procedure. All operations were carried out under a nitrogen atmosphere with oven-dried glassware. The experimental techniques used in handling air-sensitive materials are described elsewhere.[9] Tetrahydrofuran (THF) was predried over 4Å molecular sieves and distilled from benzophenone ketyl prior to use. Potassium hydride was used as received from Aldrich Chemical Company and was freed from mineral oil following the procedure in the literature.[10] α-Pinene was obtained from the Aldrich Chemical Company, (+)-3-carene was a gift from Dr. S. N. Mehra, Camphor and Allied Products, Bareilly, India, and (+)-2-carene was a gift from Dr. Sukh Dev, Malti-Chem, Baroda, India. They were distilled over a small amount of lithium aluminum hydride.

(−)-Nopol benzyl ether was purchased from Aldrich Chemical Company and used without further purification. (−)-2-Isocaranol was prepared from (+)-2-carene *via* hydroboration, followed by alkaline hydrogen peroxide ox-

idation according to a published procedure.[11] (+)-10-Benzyloxymethylisopinocampheol was prepared by a hydroboration-oxidation sequence using borane in THF, which is described in detail in a later section. The ^{11}B NMR spectra were recorded on a Varian FT-80 spectrometer and all ^{11}B chemical shifts were reported in δ(ppm) relative to $BF_3{\cdot}OEt_2$. The ^1H NMR spectra were recorded on a Varian T-60A spectrometer with Me_4Si as an internal standard and all of the chemical shifts were reported in δ(ppm) relative to Me_4Si. IR spectra were recorded on a Perkin-Elmer 1420 spectrophotometer equipped with a Perkin-Elmer 3600 IR data station. GC analyses were performed on a Hewlett-Packard 5730A instrument equipped with a Hewlett-Packard 3390A integrator/plotter using 6 ft × 0.125 in column of 10% Carbowax on Chromosorb W and an internal standard. Capillary GC analyses were performed on a Hewlett-Packard 5890 chromatograph equipped with a Hewlett-Packard 3390A integrator/plotter using 15 m Supelcowax or 50 m Methylsilicone capillary column. Optical rotations were measured on a Rudolph Polarimeter Autopol III.

Preparation of Chiral B-Alkyl-9-BBN. The procedure for the synthesis of B-(2-Icr)-9-BBN, **7**, is representative. In an oven-dried, 100-m*l*, round-bottom flask with a sidearm and an adaptor attached to a mercury bubbler were placed 3.66 g (30 mmol) of 9-BBN and 22 m*l* of THF. To the stirred slurry of 9-BBN in THF, a total of 4.09 g (30 mmol) of 2-carene $[\alpha]_D^{22}$ 92.6° (neat), [lit.[12] $[\alpha]_D^{20}$ 97.7° neat] was added and stirred 18 h at 25°C. ^{11}B NMR indicated the complete formation of **7**, δ84.8 (*br s*, THF) and the solution in THF was directly utilized for further hydridation reaction. For the preparation of B-Ipc-9-BBN, 4, NB-Enantrane™, **5**, and B-(4-Icr)-9-BBN, **6**, the corresponding (+)-α-pinene, $[\alpha]_D^{23}$ 47.26° (neat) [lit.[13] $[\alpha]_D^{20}$ 51.6°], (-)-nopol benzyl ether, $[\alpha]_D^{22}$ -26.47 (*c* 10, $CHCl_3$) [lit.[6] $[\alpha]_D^{20}$ -27.8° (*c* 10, $CHCl_3$)] and (+)-3-carene, $[\alpha]_D^{23}$ 14.9° (neat) [lit.[14] $[\alpha]_D^{23}$ 17.7° (neat)] were used. The results are summarized in Table 1.

Preparation of Chiral B-Alkoxy-9-BBN. The preparation of B-O-(2-Icr)-9-BBN, **9**, is representative. In an oven-dried, 100-m*l*, round-bottom flask with a sidearm and an adaptor attached to a gas buret were placed 3.66 g (30 mmol) of 9-BBN and 22 m*l* of THF. To the stirred slurry of 9-BBN, a total of 4.63 g (30 mmol) of (-)-2-isocaranol, α_D^{23} -30.92° (neat) [lit.[11] α_D-30.23° (neat)] was added slowly with stirring and 28.5 mmol of H_2 (95% yield) liberated within 2 h at 25°C. ^{11}B NMR indicated the complete formation of the borinic ester, **9**, δ 56.3 (*br s*, THF) and the solution was directly utilized for hydridation. For the preparation of NB-O-Enantrane, **8**, the corresponding hydroxy compound was synthesized by hydroboration of (-)-nopol benzyl ether with $BH_3{\cdot}THF$ followed by alkaline hydrogen peroxide oxidation. The (+)-10-benzyloxymethylisopinocampheol ($[\alpha]_D^{23}$ 3.2° (*c* 5.22, $CHCl_3$); > 99% GC pure; highly viscous liquid; 89% yield; ^1H NMR ($CDCl_3$) δ 7.30 (*s*, 5H), 4.52 (*s*, 2H), 1.5-4.4 (*m*, 13H), 1.23 (*s*, 3H), 0.88 (*s*, 3H); MS, m/e M^+ 274) was treated with 9-BBN following the same procedure as above. The results are summarized in Table I.

Preparation of Chiral Borohydrides. The procedure for the synthesis of K B-O-(2-Icr)-9-BBNH, **9′**, is representative. An oven-dried, 100-m*l*, round-bottom flask equipped with a Teflon stopcock on a sidearm was attached to a condenser connected to a mercury bubbler. To the flask cooled to room temperature under a stream of nitrogen was transferred potassium hydride as an oil suspension using a double-ended needle. The potassium hydride was allowed to settle and most of the oil decanted and washed with pentane (3 × 30 m*l*). To this oil-free potassium hydride (1.6 g, 40 mmol) suspended in THF (30 m*l*) was added the THF solution (30 m*l*) of **9** prepared as described earlier *via* a double-ended needele. The mixture was vigorously stirred while refluxing (65°C). The reaction was monitored by ^{11}B NMR and completed at 60 h exhibiting complete formation of **9′** δ-1.81 (*br s*, THF). Then the condenser was replaced with a tapered group-glass adaptor equipped with a stopcock and the excess potassium hydride was allowed to settle for 48 h. An aliquot of the clear supernatant was hydrolyzed in a THF-glycerine-2 *N* HCl (1:1:1) mixture and the hydrogen evolved was measured, indicating the concentration of **9′** as 0.46 *M* (92% yield); IR $\nu_{B\text{-}H}$ 2004 cm^{-1}. The solution stored over a positive pressure of nitrogen revealed no change in hydride activity and in ^{11}B NMR spectra over an extended period of time. The results for other chiral borohydrides are summarized in Table 2.

Asymmetric Reduction of Prochiral Ketones. The following procedure for the asymmetric reduction of acetophenone with **9′** is representative. The THF solution of acetophenone (5 m*l*, 5 mmol) precooled to -78°C was added to the solution of **9′** in THF (0.46 *M*, 12 m*l*, 5.5 mmol) in a 50-m*l* long-necked round-bottom flask at -78°C *via* a double-ended needle. After 72 h, unreacted hydride was destroyed by injecting *ca.* 1 equiv of precooled MeOH and stirring for 1 h at -78°C. After raising the temperature to 25°C, all the solvent and volatiles were evaporated under reduced pressure (14 mm Hg, 25°C). To the residue, 10 m*l* of EE was added and the mixture was oxidized using alkaline hydrogen peroxide. The organic layer was separated and the aqueous layer was extracted with EE (2 × 20 m*l*). The combined organic layer was washed with brine, dried ($MgSO_4$), filtered and the solvent evaporated under reduced pressure (14 mmHg, 25°C). Bulb-to-bulb distillation of the residue at 120°C bath temperature under reduced pressure (14 mmHg) yielded a mixture of product 1-phenylethanol and (-)-2-isocaranol. The distilled mixture was directly utilized for derivatization with MTPA-Cl following the established procedure in the literature.[15] Capillary GC analysis (Supelcowax, 15 *M*) of MTPA esters revealed a composition of 74.5% *R* and 25.5% *S* (*i.e.*, 49% ee). The same procedure was used for the asymmetric reduction of 3-methyl-2-butanone except that the product alcohol was separated from EE by fractional distillation using a Widmer column. Capillary GC analysis of MTPA esters of the product alcohol indicated a composition of 84.5% *R* and 15.5% *S* (*i.e*, 69% ee). In the case of K B-Ipc-9-BBNH, **4′**, the reagent solidified when cooled to -78°C in THF. Consequently, the reaction temperature of -50°C was used. Thus, acetophenone and 3-methyl-2-butanone were reduced by **4′** at -50°C with 97% and 99% yields in 24 h, by **5′** at -78°C with 92% and 95% yields in 24 h, by **6′** a at -78°C with 97% and 98% yields in 18 h, by **7′** at -78°C with 82% and 96% yields in 72 h, by **8′** at -78°C with 94% and 96% yields in 24 h, and by **9′** at -78°C with 81% and 90% yields in 72 h respectively (GC yields). All those results or asymmetric reductions are summarized in Table 3.

Acknowledgement. We are grateful to the United States Army Research Office for the financial assistance (ARO

DAAG 29-85-K-0062) and to Dr. S. N. Mehra (Camphor and Allied Products, Bareilly, India) for the gift of (+)-3-carene and to Dr. Sukh Dev (Malti-Chem, Baroda, India) for the gift of (+)-2-carene.

References and Notes

†Dedicated to Professor Nung Min Yoon on the occasion of his 60th birthday.

1. (a) Postdoctoral research associate on grant ARO DAAG 29-85-K-0062; (b) Present address: Department of Chemistry, Hallym University, Chuncheon, Republic of Korea.
2. (a) Brown, H. C.; Cha, J. S.; Nazer, B. *J. Org. Chem.* 1984, **49**, 2073; (b) Brown, H. C.; Cha, J. S.; Nazer, B.; Brown, C. A. *ibid.* 1985, **50**, 549.
3. (a) Brown, H. C. ; Park, W. S.; Cho, B. T. *J. Org. Chem.* 1986, **51**, 1934; (b) *Idem. ibid.* 3278; (c) Brown, H. C.; Cho, B. T.; Park, W. S. *ibid.* 3396.
4. Brown, H. C.; Park, W. S.; Cho, B. T.; Ramachandran, P. V. submitted for publication.
5. Krisnamurthy, S.: Vogel, F.; Brown, H. C. *J. Org. Chem.* 1977, **42**, 2534.
6. Midland, M. M.; Kazubski, A. *J. Org. Chem.* 1982, **47**, 2496.
7. Brown, H. C.; Pai, G. G. *J. Org. Chem.* 1985, **50**, 1384.
8. Tramontano, A. Ph.D. Thesis, University of California, Riverside, August 1980.
9. Brown, H. C.; Kramer, G. W.; Levy, A. B.; Midland, M. M. "Organic Synthesis via Boranes"; Wiley-Interscience: New York, 1975.
10. Brown, C. A. *J. Org. Chem.* 1974, **39**, 3913.
11. Acharya, S. P. Brown, H. C. *J. Am. Chem. Soc.* 1967, **89**, 1925.
12. Ohloff, G.; Schulte-Elte, K. H.; Giersch, W. *Helv. Chim. Acta* 1965, **48**, 1665.
13. Brown, H. C.; Singaram, B. *J. Org. Chem.* 1984, **49**, 945.
14. Jadhav, P. K.; Vara Prasad, J. V. N.; Brown, H. C. *J. Org. Chem.* 1985, **50**, 3203.
15. Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* 1973, **95**, 512.

Chemical Modification of Glycolate Oxidase from Spinach by Diethyl Pyrocarbonate. Evidence of Essential Histidine for Enzyme Activity†

Kun Kook Lee, Hong Sun Kim, and Jung Do Choi*

Department of Chemistry, Chungbuk National University, Cheongju 310

Received March 31, 1987

FMN-dependent glycolate oxidase from spinach is inactivated by diethyl pyrocarbonate at pH 7.0. Inactivation of both apo- and holoenzyme by diethyl pyrocarbonate follows pseudo-first-order kinetics and first order with respect to the reagent. A series of difference spectra of inactivated and native enzymes show a single peak at 240 nm, indicating the modification of histidyl residues. No decrease in absorbance at around 280 nm due to formation of O-carbethoxytyrosine is observed. The rate of inactivation is dependent on pH, and the data for pH dependent rates implicate the involvement of a group with a pKa of 6.9. The activity lost by treatment with diethyl pyrocarbonate could be almost fully restored by incubation with 0.75M hydroxylamine. The reactivation by hydroxylamine and the pH dependence of inactivation are also consistent with that the inactivation is due to modification of histidyl residues. Although coenzyme FMN is without protective effect, the substrate glycolate, the product glyoxylate, and two competitive inhibitors, oxalate and oxalacetate, provide marked protection against the inactivation of the holoenzyme. These results suggest that the inactivation of the oxidase by diethyl pyrocarbonate occurs by modification of essential histidyl residue(s) at the active site.

Introduction

Glycolate oxidase (glycolate: oxygen oxidoreductase, EC 1.1.3.1) catalyzes the conversion of glycolate to glyoxylate, with O_2 as electron acceptor. The oxidase is found in the peroxisomes of mammalian liver and kidney,[1] and also in the green leaves of plants.[2] The enzyme of mammalia is probably involved in the metabolic production of oxalate by the oxidation of glycolate through glyoxylate.[3] Inhibition studies of mammalian enzyme have been made extensively,[1,4,5] since inhibition of the enzyme might be useful for treatment of hyperoxaluria.

In green plants, glycolate oxidase is one of the key enzymes involved in the process of photorespiration.[2] The primary reaction of photorespiration is the production of glycolate from ribulose-1,5-diphosphate and O_2, and the first step in glycolate pathway is the oxidation of glycolate to glyoxylate. Since photosynthetic productivity is drastically reduced by active photorespiration in C-3 plants, an effective control of photorespiration might be one possible way to increase the efficiency of photosynthesis. It was shown that inhibition of photorespiration with biochemical inhibitors of glycolate synthesis[6] or oxidation[7] increase net photosynthesis by 50% or more for a short period.

It is necessary to elucidate the nature and role of the amino acid residues within the active site for rational design of specific inhibitors of the oxidase. Recently. Lee and Choi[8] confirmed that an arginyl residue at the active site is involved

† Dedicated to Professor Nung Min Yoon on the occasion of his 60th birthday.