0.34 mmol of hydrogen (110%) had been absorbed (*ca.* 30 sec). Qualitative glpc analysis (15% FFAP on Chromosorb W, 8 ft × 0.125 in., 94°) showed one major and five minor products. The major product, which was collected by preparative glpc (20% SE-30 on Chromosorb W, 10 ft × 0.375 in., 110°), showed an nmr spectrum identical with that published for **3**:[18] nmr ($CDCl_3$) δ 5.80 (m, 2 H), 3.1 (br m, 3 H), 2.69 (br m, 1 H), 2.2–1.2 (series of m, 6 H).

7,8-Dichlorobicyclo[4.2.0]octa-2,4-diene. This dichloride was prepared in 54% yield from cyclooctatetraene and chlorine by the previously described method: bp 102–104° (2 mm);[30] nmr ($CDCl_3$) δ 5.2 (m, 4 H), 4.67 (t, 1 H), 4.45 (t, 1 H), 3.5 (br m, 1 H), 3.0 (br m, 1 H). On the basis of the nmr spectrum, our compound appears to be the *trans*-7,8-dichloro isomer.[31]

7,8-Dichlorobicyclo[4.2.0]oct-2-ene (4). A solution of 9.6 g (0.055 mol) of 7,8-dichlorobicyclo[4.2.0]octa-2,4-diene in 150 ml of a 50:50 methanol–ethyl acetate mixture and 50 mg of 5% palladium on carbon was partially reduced in a Parr shaker. The shaker was stopped when 0.055 mol of hydrogen had been absorbed (*ca.* 5 min). The solution was filtered, the solvent was removed, and the residue was distilled to give 6.8 g (73%) of **4**, bp 115–116° (30 mm). An analytical sample was obtained by preparative glpc (20% SE-30 on Chromosorb W, 20 ft × 0.25 in.): nmr ($CDCl_3$) δ 5.94 (m, 2 H), 4.7–3.9 (9-line m, 2 H), 3.4–1.3 (series of m, 6 H).

Anal. Calcd for $C_8H_{10}Cl_2$: C, 54.29; H, 5.65; Cl, 40.06. Found: C, 54.48; H, 5.85; Cl, 39.89.

Bicyclo[4.2.0]octa-2,7-diene (5). A 0.85-g (0.037 g-atom) sample of freshly cut sodium was added to 200 ml of ammonia. To this stirred solution under a nitrogen atmosphere was added 1.5 g (0.008 mol) of **4** in 100 ml of dry ether. The reaction solution was stirred for 1.5 hr and then was quenched with ammonium chloride. After this, 400 ml of water was added and the mixture was continuously extracted with ether. The ether extract was dried ($MgSO_4$) and the solvent was removed by careful distillation, leaving 0.74 g (83%) of **5** which was >97% pure by glpc (20% SE-30 on Chromosorb W, 10 ft × 0.125 in., 70°): nmr (C_6D_6) δ 6.08 (d, 1 H), 5.9 (m, 3 H), 3.2 (br m, 2 H), 2.3–1.2 (series of m, 4 H).[19,20]

Reaction Scope Studies. The vicinal dihalides 1,2-dichlorohexane, 1,2-dichlorocyclohexane, 1,2-dichlorocyclooctane, and 1,2-dibromocyclooctane were prepared in the usual way by addition of halogen to the corresponding alkene at low temperature.[32] In all cases the dihalides were purified and had physical and spectral properties in agreement with the indicated structures. Dehalogenations were carried out by the procedure given above for the formation of **5**. The conversion of dihalide to alkene was quantitatively measured by glpc using appropriate *n*-alkane internal standards and detector response factors obtained from standardized solutions.

Acknowledgment. We thank Mr. Richard E. Hurst for expert help in the reaction scope studies.

Registry No.—1, 41326-65-2; **2**, 50987-22-9; **3**, 31750-01-3; **4**, 50987-23-0; **5**, 3786-98-9; benzene, 71-43-2; *cis*-3,4-dichlorocyclobutene, 2957-95-1; cyclooctatetraene, 629-20-9; *trans*-7,8-dichlorobicyclo[4.2.0]octa-2,4-diene, 34719-15-8.

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Cleavage of Protecting Groups with Boron Tribromide

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Boron halides have been used for the cleavage of methyl ethers,[1,2] benzhydryl esters,[3] *tert*-butyloxycarbonyl amine protecting groups,[4,5] and hindered esters.[6] A recent report[7] that benzyloxycarbonyl amine protecting groups can be removed quantitatively with boron tribromide prompts us to report on our observations using this reagent in peptide chemistry. We observed that, in addition to the removal of *N*-*tert*-butyloxycarbonyl and *N*-benzyloxycarbonyl protecting groups, boron tribromide in methylene chloride gave rapid conversion of methyl, ethyl, *tert*-butyl, benzyl, and *p*-nitrobenzyl esters to their corresponding acids. The alkaline conditions usually employed to hydrolyze methyl and ethyl esters enhances the chances of racemization. The sensitivity of the *N*-benzyloxycarbonyl group[8] and the seryl peptide bond[9-11] to strongly basic conditions also render that method unattractive for general usage.

The products after boron tribromide treatment were isolated by ion-exchange chromatography, found to be analytically pure, and were obtained in yields of 60–90% after crystallization. Optical purity of the products was ascertained to be >99.9% using the procedure of Manning and Moore.[12] Table I summarizes the results obtained for the deprotection of a variety of substrates with boron tribromide. Many of the widely used amino acid side chain protecting groups[13] [Ser(Bzl), Tyr(Bzl), Tyr(Cl_2Bzl), Thr(Bu^t), Glu(OMe), Glu(OEt), Glu(OBzl), Asp(OBu^t), and Lys(Z)] were also removed by boron tribromide, whereas certain other groups [Arg(Tos), Cys(Bzl), and His(im-Bzl)] were unaffected. Although Arg(Tos) was not

Table I
Deprotection with 1.0 *M* BBr_2 (CH_2Cl_2)

Substrate	Registry no.	Product	Yield, %[a]	Yield, %[b]	Optical rotation, deg $[\alpha]^{25}D$ found / standard
					26.64 (*c* 1, 6 *M* HCl)
Z-Val-OH	1149-26-4	Val	86.5	71.5	27.50 (*c* 1, 6 *M* HCl)
					14.14 (*c* 2.1, 6 *M* HCl)
Boc-Leu-OH	13139-15-6	Leu		62.6	14.99 (*c* 2.2, 6 *M* HCl)
					13.70 (*c* 2.3, 6 *M* HCl)
Z-Leu-OMe	51021-87-5	Leu		61.0	14.99 (*c* 2.2, 6 *M* HCl)
Z-Glu(OMe)-OH	4652-65-7	Glu	99.7		
					29.13 (*c* 1.0, 6 *M* HCl)
Z-Glu(OBzl)-OH	5680-86-4	Glu	85.5	79.5	28.06 (*c* 1.0, 6 *M* HCl)
Z-Asp(OBu^t)-OH	5545-52-8	Asp	94.8		
					31.10 (*c* 1.0, 6 *M* HCl)
H-Glu(OEt)-OEt	16450-41-2	Glu		87.9	28.06 (*c* 1.0, 6 *M* HCl)
					28.20 (*c* 1.1, 6 *M* HCl)
H-Glu(OBzl)-OBzl[c]	2768-50-5	Glu	100	79.8	28.06 (*c* 1.0, 6 *M* HCl)
H-Val-OMe	4070-48-8	Val	82.7		
H-Val-OBu^t	13211-31-9	Val	94.2		
					6.97 (*c* 4.0, 6 *M* HCl)
Boc-Tyr(Bzl)-OH	2130-96-3	Tyr	81.4	76.2	7.62 (*c* 4.0, 6 *M* HCl)
Boc-Tyr(Cl_2Bzl)-OH	40298-71-3	Tyr	94.2		
					14.79 (*c* 4.4, 1 *M* HCl)
Boc-Ser(Bzl)-OH	23680-31-1	Ser	74.0	64.3	14.10 (*c* 9.0, 1 *M* HCl)
					−14.95 (*c* 1.0, 1 *M* HCl)
Z-Thr(Bu^t)OBzl(*p*-NO_2)	16879-87-1	Thr	78.5	74.3	−15.26 (*c* 1.0, 1 *M* HCl)
Z-Lys(Z)-OH	51021-86-4	Lys	99.8		
					−28.84 (*c* 0.5, H_2O)
Z-Trp-OH	7432-21-5	Trp	72.2	58.3	−30.88 (*c* 0.5, H_2O)
Z-His-OH	31008-76-1	His	89.1		
					24.71 (*c* 1.0, 1 *M* HCl)
Z-Met-OH	1152-62-1	Met	77.6	62.7	24.03 (*c* 1.0, 1 *M* HCl)
Boc-Cys(Bzl)-OH	5068-28-0	Cys(Bzl)	96.0		
					−20.37 (*c* 1.0, 1 *M* HCl)
Z-Ala-Leu-OEt	41041-70-7	Ala-Leu		73.5	−21.61 (*c* 1.0, 1 *M* HCl)
					−20.80 (*c* 1.0, 1 *M* HCl)
Z-Ala-Leu-OBzl	51021-85-3	Ala-Leu		92.1	−21.61 (*c* 1.0, 1 *M* HCl)

[a] By amino acid analysis of reaction mixture. [b] After recrystallization as analytically pure product. [c] Dissolved in a mixture of *N*,*N*-dimethylacetamide–CH_2Cl_2 (6:40).

cleaved by boron tribromide, Arg(NO_2) underwent partial deprotection and gave a mixture of Arg, Orn, and Arg(NO_2). Treatment of Z-Met, Z-Trp, and Boc-Tyr(Bzl) (without addition of scavenging reagents) gave the corresponding amino acids free of alkylated side products. Since methionine was reported to react slowly with boron tribromide,[14] quantitative amino acid analysis was performed on the crude product from the reaction of Z-Met with boron tribromide. No evidence for any ninhydrin-positive side products with the free methionine was observed. Therefore the mild conditions employed for the deprotection caused no secondary reaction of methionine.

Deprotection of derivatives of asparagine and glutamine with boron tribromide resulted in partial degradation to aspartic acid and glutamic acid. Treatment of Z-Asn-OH with boron tribromide gave a mixture of Asn (95.8%) and Asp (4.2%). Similar treatment of Z-Gln-OH afforded Gln (90.1%) and Glu (9.9%). The reaction of Z-Asn-OMe and Z-Gln-OMe with boron tribromide gave respective mixtures of asparagine–aspartic acid and glutamine–glutamic acid. There was no evidence for isoasparagine or isoglutamine and it was concluded that there was no intermediate formation of Z-L-aminosuccinimide or Z-L-α-aminoglutarimide by the new procedure as previously postulated[15] for the alkaline hydrolysis of Z-Asn-OMe and Z-Gln-OMe.

Treatment of Z-Ala-Leu-OBzl or Z-Ala-Leu-OEt with boron tribromide gave the free peptide, Ala-Leu, exclusively. In each case there was no evidence for the presence of Ala or Leu and it was concluded that the peptide bond is unaffected by the reagent. The boron tribromide deprotection reactions were generally carried out in methylene chloride. *N*,*N*-Dimethylacetamide was found to serve as a satisfactory cosolvent with methylene chloride for the deprotection of insoluble substrates.

Experimental Section

Boron tribromide was purchased from Ventron Corp., Beverly, Mass., and was used without further purification. Solutions of 1.0 *M* boron tribromide in methylene chloride were stored in a Teflon bottle, placed into a larger container containing Drierite, and kept at −20°. *N*-Benzyloxycarbonylamino acids and other amino acid derivatives were synthesized or purchased from Fox Chemical Co. and examined for purity by thin layer chromatography prior to usage. All amino acid derivatives used were of the L configuration. *N*,*N*-Diemthylacetamide (spectrophotometric grade) was purchased from Aldrich Chemical Co., Milwaukee, Wis., and dried over molecular sieve. All other reagents and solvents were of reagent grade and used without further purification. C, H, N microanalyses were determined to within ±0.4 of the theoretical values. Optical rotations were measured in a jacketed 1-dm cell on a Perkin-Elmer Model 141 polarimeter. Thin layer chromatography was performed on all amino acids and peptides using silica gel G in three separate systems and developed with fluorescamine.[16] [BuOH–AcOH–EtOAc–H_2O (1:1:1:1); BuOH–AcOH–H_2O (4:1:1); BuOH–AcOH–pyridine–H_2O (15:3:10:12)]. The crude amino acids and peptides following boron tribromide treatment were chromatographed on AG-50WX2 (Bio-Rad Laboratories, Richmond, Calif.). The resin was packed in a column (30 × 4.5 cm), regenerated with 2 *M* NaOH, H_2O, 2 *M* HCl, and H_2O, and equilibrated and eluted with 0.4 *M* pyridine acetate, pH 4.0. Amino acid analyses were performed on the Joel Model JLC-5AH amino acid analyzer.

Procedure for Protecting-Group Cleavage. The substrate (2.0 mmol) was dissolved in CH_2Cl_2 (50 ml) and cooled to −10° and 10 ml of 1 *M* BBr_3 in CH_2Cl_2 (10.0 mmol) added dropwise with stirring. Stirring continued at −10° for 1 hr and at 25° for 2 hr. The reaction was terminated by careful dropwise addition of

water (50 ml). The layers were separated, the organic phase was washed with H_2O (3 × 25 ml), and the combined aqueous layers were evaporated to dryness. The residue was taken up in H_2O and chromatographed on AG-50WX2 using 0.4 *M* pyridine acetate (pH 4.0) as eluent. In several cases the buffer was adjusted to higher pH in order to elute the product in a volume of 375–475 ml. The ninhydrin-positive fractions were pooled, lyophilized, and crystallized.

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Registry No.—Boron tribromide, 10294-33-4.

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Oxidation of Tyrosine and of NH_2-Terminal Tyrosine Peptides with the Cu^{2+}/H_2O_2 System

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The oxidations of tyrosine and of tyrosine-containing peptides to aminochromes have long been known as enzymatic reactions.[2] More recently, analogous chemical oxidations have been studied spectroscopically. Wilchek, *et al.*,[3] reported that, at room temperature, *N*-bromosuccinimide oxidation of tyrosine esters and of tyrosinamide (but not of free tyrosine) gave a product that was identified spectroscopically as an unstable red aminochrome, λ_{max} 480 and 320 nm. Dukler, *et al.*,[4] found that tyrosine methyl ester and di- and tripeptides with NH_2-terminal tyrosine were oxidized at room temperature by potassium nitrosodisulfonate (Fremy salt), forming a product with absorption maxima at 305 and 475 nm, characteristic for dopachrome (**2**, R = H). As in the case of the enzymatic reaction, oxidation by this reagent of peptides with COOH-terminal tyrosine resulted, not in an aminochrome, but in dopaquinone, indicated by the characteristic *o*-quinone absorption at 390 nm. Dukler, *et al.*, did not report on the oxidation of tyrosine itself, but found that carbobenzoxy-L-tyrosine gave, on short-term treatment with Fremy salt followed by treatment with $Na_2S_2O_4$ and cleavage of the carbobenzoxy moiety, 3,4-dihydroxy-L-phenylalanine; longer term treatment with Fremy salt gave polymeric oxidation products of tyrosine.

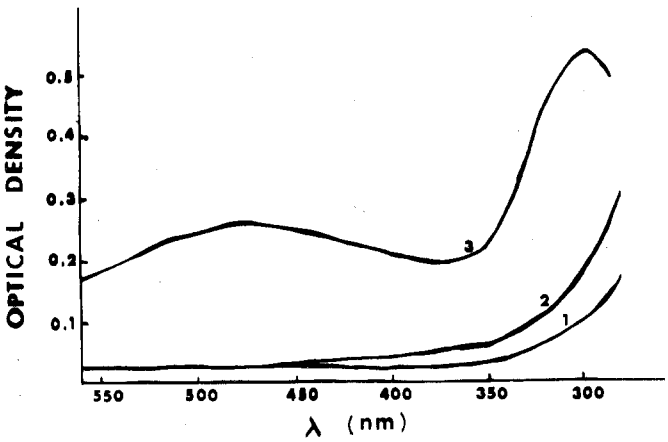
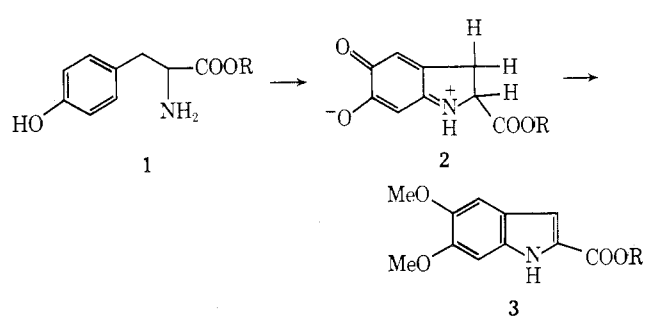


Figure 1. Oxidation of tyrosine by the Cu^{2+}/H_2O_2 system: curve 1, zero time; curve 2, after 16 hr at room temperature; curve 3, after 16 hr at room temperature, followed by addition of Pt black. No change in curve 3 was observed after 8 hr at room temperature.

We wish to report the effect of another oxidizing system, Cu^{2+}/H_2O_2 (3% unstabilized H_2O_2 containing trace amounts of Cu^{2+}), on tyrosine and on some NH_2-terminal and COOH-terminal tyrosine peptides, and the first direct nonenzymatic conversion of free tyrosine to an aminochrome. This metal-activated hydrogen peroxide system contains hydroxy and peroxy radicals, and oxidations by this system are considered to proceed by radical mechanisms.

Results and Discussion

Tyrosine. Tyrosine (**1**, R = H) was treated at room temperature with excess Cu^{2+}/H_2O_2 reagent, and the ultraviolet absorption spectrum was scanned at intervals against a Cu^{2+} blank of the same concentration. No evidence for dopachrome formation was obtained, even after 16 hr at room temperature. A predominant end absorption at shorter wavelengths was observed; the reagent and unreacted tyrosine are known to absorb in this region (Figure 1, curves 1 and 2).



Addition of Pt black at the end of the 16-hr period caused the immediate development of two absorption maxima at 305 and 475 nm, characteristic of dopachrome (**2**, R = H) (Figure 1, curve 3). These maxima did not change with time or with addition of more H_2O_2. In the absence of Cu^{2+} from the peroxide system, Pt black did not show this effect.