Anal. Calcd. for $C_{20}H_{42}BO_2Br$: C, 59.31; H, 10.38; B, 2.67; Br, 19.73. Found: C, 59.64; H, 10.43; B, 2.95; Br, 19.47.

Eliminations.—Treatment of 0.28 g. of dibutyl-2-bromoethaneboronate with 10 ml. of water yielded 96% of ethylene, confirmed by infrared comparison with an authentic sample. Treatment of 0.5 g. of dibutyl 2-bromoethaneboronate with 0.6 g. of sodium thiocyanate in 3 ml. of acetone yielded 90% of ethylene in 2 hr. Tributyl borate (identified by infrared) and an unstable oil were isolated from the solution. The oil was only slightly soluble in butyl borate, but appeared to separate slowly over a period of several days. The infrared spectrum was consistent with the presence of thiocyano or isothiocyano groups. Extensive decomposition occurred on attempted distillation. Bis(diisobutylcarbinyl) 2-bromoethaneboronate underwent similar elimination in the presence of sodium thiocyanate to yield tris(diisobutylcarbinyl) borate,[6] m.p. 102°, further confirmed by microanalysis. In the other eliminations mentioned in the discussion section, evolution of gas on mixing and isolation of butyl borate from the reaction mixture were considered sufficient evidence that elimination was occurring.

Acetylation of Serine during Bradykinin Synthesis

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In a previous report[1], we described two carbobenzoxypentapeptide intermediates obtained during the synthesis of bradykinin which did not appear to be identical, but which gave the same carbobenzoxyhexapeptide on further reaction. The pentapeptides were obtained by different procedures. In the first case carbobenzoxy-L-phenylalanine *p*-nitrophenyl ester reacted with L-seryl-L-prolyl-L-phenylalanylnitro-L-arginine methyl ester and secondly, carbobenzoxy-L-phenylalanyl-L-seryl azide reacted with L-prolyl-L-phenylalanylnitro-L-arginine methyl ester. Both of the pentapeptides were crystalline but differed in melting point by 65° and in rotation by 15°. The infrared curves of the two compounds were not significantly different enough to confirm any structural anomalies. X-Ray diffraction patterns of the two peptides revealed dissimilarities which could possibly be due to different crystalline forms, but seeding a solution of one of the pentapeptides with the other failed to induce crystallization.

The solution to the problem of the two pentapeptides came with the attempted preparation of the O-acetyl analog of bradykinin. The intermediate carbobenzoxy 6-O-acetylserine pentapeptide was found to possess the physical properties of the pentapeptide obtained by the one plus four coupling. O-Acetyl analysis confirmed the presence of this functional group in both compounds while the pentapeptide prepared by the two plus three reaction did not contain an O-acetyl. Reexamination of the other bradykinin intermediates from the hexapeptide to the tricarbobenzoxynonapeptide also revealed the presence of O-acetyl groups. The results are given in Table I. The acetate group is undoubtedly lost during hydrolysis of the protecting methyl ester since it does not appear in any of the products after this step.

The O-acetyl group on serine probably is introduced during the hydrobromine acid–acetic acid cleavage of the carbobenzoxy group since this side reaction has been reported previously.[2] The consequences of this reaction are usually not troublesome and in practice we have found that the products containing this group are higher melting and more easily crystallized than peptides without it. Care should be exercised, however, in preparing peptides containing serine in which a step involving hydrolysis with alkali is not involved.

Experimental[3]

Carbobenzoxy-O-acetyl-L-seryl-L-propyl-L-phenylalanylnitro-L-arginine Methyl Ester.—To a cold (5°) solution of 4.3 g. (0.0078 mole) of L-propyl-L-phenylalanylnitro-L-arginine methyl ester hydrobromide[1] in 50 ml. of dimethylformamide was added 1.5 g. of triethylamine. After 5 min., the precipitate was removed by filtration; to the filtrate was added 3.2 g. (0.078 mole) of N-carbobenzoxy-O-acetyl-L-serine *p*-nitrophenyl ester.[4,5] The yellow solution was kept 18 hr. at 25°, diluted with 250 ml. of ethyl acetate, and washed with water, aqueous 5% sodium carbonate, water, dilute hydrochloric acid, dried, and evaporated. Ether was added giving a white solid which was recrystallized from methanol–ether, 4 g. (70%), m.p. 166–168°, $[\alpha]^{23}D$ −55.7° (*c* 1.06, dimethylformamide); reported[5] m.p. 170–172°, $[\alpha]^{20}D$ −56.6° (*c* 1.06, dimethylformamide).

Anal. Calcd. for $C_{34}H_{44}N_8O_{11}$: C, 55.12; H, 5.98; N, 15.12. Found: C, 54.96; H, 6.17; N, 15.12.

Carbobenzoxy-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanylnitro-L-arginine Methyl Ester.—To a cold (10°) solution of the carbobenzoxytetrapeptide, 7 g. (0.0095 mole), in 100 ml. of glacial acetic acid was added 6 g. of anhydrous hydro-

TABLE I

BRADYKININ INTERMEDIATES

		Formula	Calcd. % C	H	N	O-Ac	Found, % C	H	N	O-Ac
1.	CBZ-L-Phe-O-Ac-L-Ser-L-Pro-L-Phe-NO_2-L-Arg-OCH_3	$C_{43}H_{53}N_9O_{12}$	58.17	6.02	14.20	4.85	58.05	6.06	14.83	5.9
2.	CBZ-Gly-L-Phe-O-Ac-L-Ser-L-Pro-L-Phe-NO_2-L-Arg-OCH_3	$C_{45}H_{56}N_{10}O_{13}$	57.20	5.97	14.83	4.56	57.43	6.11	15.00	4.15
3.	CBZ-L-Pro-Gly-L-Phe-O-Ac-L-Ser-L-Phe-NO_2-L-Arg-OCH_3	$C_{50}H_{63}N_{11}O_{14}$	57.62	6.09	14.78	4.12	57.34	6.10	15.24	3.50
4.	CBZ-L-Pro-L-Pro-Gly-L-Phe-O-Ac-L-Ser-L-Pro-L-Phe-NO_2-L-Arg-OCH_3	$C_{55}H_{70}N_{12}O_{15}$	57.99	6.19	14.76	3.92	57.66	6.13	14.98	4.23
5.	TRICBZ-L-Arg-L-Pro-L-Pro-Gly-L-Phe-O-Ac-L-Ser-L-Pro-L-Phe-NO_2-L-Arg-OCH_3	$C_{77}H_{94}N_{16}O_{20}$	59.14	6.06	14.34	2.75	58.92	6.14	14.66	3.24

(1) E. D. Nicolaides and H. A. DeWald, *J. Org. Chem.*, **26**, 3872 (1961).

(2) St. Guttmann and R. A. Boissonnas, *Helv. Chim. Acta*, **41**, 1852 (1958).

(3) Melting points were taken using a Thomas–Hoover capillary melting point apparatus and are corrected.

(4) H. A. DeWald and E. D. Nicolaides, to be published.

(5) M. A. Ondetti, *J. Med. Chem.*, **6**, 10 (1963).

gen bromide. The solution was kept 2 hr. at 25°, poured into 1 l. of dry ether, and the precipitate removed, washed with ether, and dried *in vacuo*. The resulting crude hydrobromide salt of the tetrapeptide, 9 g., was dissolved in 50 ml. of dimethylformamide, cooled to 0°, and 4 g. of triethylamine added. After 5 min., the solid was removed and 4.5 g. (0.0107 mole) of carbobenzoxy-L-phenylalanine *p*-nitrophenyl ester was added to the filtrate. The yellow solution was stirred 18 hr. at 25°, diluted with 250 ml. of ethyl acetate; the solution was washed with water, aqueous 5% sodium carbonate, water, and dilute hydrochloric acid. The solution was dried over magnesium sulfate, evaporated to a small volume, and ether added. The solid was removed and recrystallized from dimethylformamide–ether; yield 5 g. (50%), m.p. 215–216°, $[\alpha]^{23}D$ −60° (*c* 1.1, dimethylformamide); reported[5] m.p. 214–216°, $[\alpha]^{20}D$ −57° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{43}H_{53}N_9O_{12}$: C, 58.17; H, 6.02; N, 14.20; O-Ac, 4.85. Found: C, 58.10; H, 6.17; N, 14.29; O-Ac, 4.55.

Carbobenzoxyglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanylnitro-L-arginine Methyl Ester.—The carbobenzoxy group was removed from 3.5 g. (0.004 mole) of the pentapeptide with hydrobromic acid–acetic acid. The crude product was dissolved in 50 ml. of dimethylformamide, cooled to 5°, and 1 g. of triethylamine added. The precipitate was removed and 1.5 g. (0.0045 mole) of carbobenzoxyglycine *p*-nitrophenyl ester was added to the filtrate. After 3 days at room temperature, the solution was diluted with four volumes of ethyl acetate giving a white solid which was recrystallized from methanol–ether; 3 g. (79%), m.p. 222–224°, $[\alpha]^{23}D$ −55.8° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{45}H_{56}N_{10}O_{13}$: C, 57.20; H, 5.97; N, 14.83; O-Ac, 4.56. Found: C, 57.19; H, 5.96; N, 14.92; O-Ac, 4.20.

Penicillin Sulfones

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We wish to report an improved procedure for preparing penicillin sulfones. Recent syntheses[1,2] have required that the carboxyl group be esterified during oxidation, and the protecting group be subsequently removed by hydrogenolysis. Using potassium permanganate in neutral aqueous solution, we prepared the sulfones of benzylpenicillin, 2,6-dimethoxyphenylpenicillin (methicillin[3]), 5-methyl-3-phenyl-4-isoxazolylpenicillin (oxacillin[3]), D-(−)-α-aminobenzylpenicillin (ampicillin[3]), and *dl*-α-phenoxyethylpenicillin (phenethicillin[3]) by direct oxidation of their salts or free acids. Ampicillin was oxidized as its N-carbobenzyloxy derivative which yielded ampicillin sulfone on hydrogenolysis.

The products were easily isolated and purified in satisfactory yields. Infrared spectra of the sulfones showed the characteristic shift of the β-lactam band from 5.6 to 5.50–5.53 μ, and the appearance of bands at 7.6 and 8.9 μ due to the sulfone group.

Experimental[4]

The sulfones prepared in this work, their physical constants, elemental analyses, and other data are listed in Table I.

General Procedure for the Preparation of Penicillin Sulfones.—The appropriate penicillin salt or free acid (0.035 mole) was added to 180 ml. of water, the pH was adjusted to 7.0–7.5, and the resulting solution was cooled to 0–5°. A solution of 5.5 g. (0.035 mole) of potassium permanganate, 1.80 ml. of 85% phosphoric acid (specific gravity 1.70), and 140 ml. of water was added to the penicillin solution at such a rate as to keep the temperature below 10°. The pH was maintained between 6.0 and 7.5 using 5–10% aqueous sodium hydroxide or 10% phosphoric acid. About 10 min. after the addition, excess potassium permanganate was destroyed with sodium bisulfite, if necessary. The manganese dioxide was removed by passing the mixture through a Dicalite-precoated filter, and the pH of the cooled filtrate was then slowly adjusted to 2.0–2.3 with 10% phosphoric acid. The penicillin sulfone-free acid which precipitated was collected and washed with cold water. It was usually dried by storing overnight in a vacuum desiccator over Drierite.

Two compounds needing special comment are described.

D(−)-α-Aminobenzylpenicillin Sulfone.—D(−)-α-N-Carbobenzyloxyaminobenzylpenicillin sulfone, 7.5 g. (0.0146 mole) was added to 70 ml. of water and dissolved by adjusting the pH to 6.5 with 10% sodium hydroxide. The previous solution was added to 7.5 g. of prehydrogenated 30% palladium-on-diatomaceous earth catalyst in 25 ml. of water and shaken under 48 p.s.i.g. of hydrogen for 2.25 hr. Methyl isobutyl ketone (50 ml.) was added, the

(1) A. W. Chow, N. M. Hall, and J. R. E. Hoover, *J. Org. Chem.*, **27**, 1381 (1962).

(2) E. Guddal, P. Morch, and L. Tybring, *Tetrahedron Letters*, **9**, 381 (1962).

(3) The trade-marks of Bristol Laboratories, a division of Bristol-Myers Co., for methicillin, oxacillin, ampicillin, and phenethicillin are, respectively, Staphcillin, Prostaphlin, Polycillin, and Syncillin.

(4) All melting points are corrected. Microanalyses were performed by Richard M. Downing, and the infrared measurements were performed by David F. Whitehead.

TABLE I

PENICILLIN SULFONE FREE ACIDS

Penicillin sulfone	Molecular formula	Yield, %	M.p., °C. dec.	Recrystallization solvent	Analyses—Calcd. % C	Analyses—Calcd. % H	Analyses—Found % C	Analyses—Found % H
Benzyl	$C_{16}H_{18}N_2O_6S$	84–87	123.0–124.0	Ethyl acetate and petroleum ether	52.46	4.95	52.70	5.14
2,6-Dimethoxyphenyl	$C_{17}H_{20}N_2O_8S$	55–70	174.5–174.8	Ethyl acetate and petroleum ether	49.51	4.89	49.45	5.30
5-Methyl-3-phenyl-4-isoxazolyl	$C_{19}H_{19}N_3O_7S$	35–61	132.0–134.0	Water	52.65	4.42	52.74	4.49
D-(−)-α-N-Carbobenzyloxyaminobenzyl-hemihydrate[a]	$(C_{24}H_{25}N_3O_8S)_2H_2O$	30–42	115.0–116.5	Ethyl acetate and petroleum ether	55.00	4.99	55.20	4.67
D-(−)-α-Aminobenzyl	$C_{16}H_{19}N_3O_6S$	30	228.6–229.4	Aerosol OT-methyl isobutyl ketone	50.39	5.02	50.80	5.05
dl-α-Phenoxyethyl(*sym*-dibenzylethylenediamine salt)	$C_{60}H_{60}N_6O_{14}S_2$	60	118.0–118.3	Methyl isobutyl ketone	58.05	5.81	57.70	5.86

[a] Calcd. for water: 1.7%. Found: 1.6%