2,5-Bisbenzylamino-1,4-benzoquinone. To 0.1 mole of butyl nitrite and 0.2 mole of benzylamine, 0.01 mole of hydroquinone was added; it was expected that the reaction would be inhibited if there were a free radical mechanism. However, a vigorous reaction began immediately; there was copious evolution of gas, and the appearance in the solution of an intense red color not previously observed. After a short time a red solid precipitated; it was found to be insoluble in all the usual solvents except glacial acetic acid, from which it was recrystallized as microscopic scarlet prisms, m.p. 259°, (lit. 246°)[11] not depressed by 2,5-bisbenzylamino-1,4-benzoquinone prepared from benzylamine and *p*-quinone in alcoholic solution.

Anal.[26] Calc'd for $C_{20}H_{18}N_2O_2$: C, 75.45; H, 5.70; N, 8.80. Found: C, 75.52; H, 5.67; N, 9.09.

DEPARTMENT OF APPLIED CHEMISTRY
ROYAL MELBOURNE TECHNICAL COLLEGE
MELBOURNE, VICTORIA, AUSTRALIA

(26) Microanalysis by Dr. K. W. Zimmerman and staff, Organic Microanalytical Laboratory, Commonwealth Scientific and Industrial Research Organization, Melbourne, Victoria.

The Preparation of L-Arginine Dipeptides

CASIMIR BERSE AND LUCIEN PICHE

Received March 12, 1956

The synthesis of peptides containing L-arginine has been delayed by the lack of appropriate methods to cover its highly polar guanidino group under conditions that must be reversible in the presence of the newly formed peptide link. The preparation of a series of dipeptides containing L-arginine was recently described[1,2] in which α-carbobenzoxy-ω-nitro-L-arginine was used as an intermediate in the mixed anhydride method of condensation; Anderson[3] used the pyrophosphite method.

We wish to submit that *p*-nitrocarbobenzoxy chloride is a more satisfactory covering agent of ω-nitro-L-arginine in providing better yields and generally more crystalline intermediates. It has already been used by Gish and Carpenter to cover the α-amino group of L-arginine acid chloride, the guanidino group being either also covered by the *p*-nitrocarbobenzoxyl group[4] or considered sufficiently stable as a zwitterion to protect it against undesired condensations.[5] Both methods have been found to be impractical, cyclization products of substituted L-arginine being formed in the early stages of condensation reactions.

(1) Hofmann, Peckham, and Rheiner, *J. Am. Chem. Soc.*, **78**, 238 (1956).

(2) Van Orden and Smith, *J. Biol. Chem.*, **208**, 751 (1954).

(3) Anderson, *J. Am. Chem. Soc.*, **75**, 6081 (1953).

(4) Gish and Carpenter, *J. Am. Chem. Soc.*, **75**, 950 (1953).

(5) Gish and Carpenter, *J. Am. Chem. Soc.*, **75**, 5872 (1953).

α-*p*-Nitrocarbobenzoxy-ω-nitro-L-arginine was combined as a mixed anhydride with aniline and with the ethyl esters of the following amino-acids: glycine, L-phenylalanine, L-leucine, L-tyrosine, β-phenyl-L-serine and L-glutamic acid.

Saponification of the resulting ω-nitro-α-*p*-nitrocarbobenzoxy-L-arginyl peptide ethyl esters with NaOH gave the corresponding substituted dipeptides, the hydrogenation of which, with 10% palladium catalyst on carbon gave the dipeptides (or their acetates, in acetic solution).

In preparing ω-nitro-α-*p*-nitrocarbobenzoxy-L-arginyl-L-phenylalanine, we were able to couple the mixed anhydride directly with L-phenylalanine. The resulting product was identical with the compound obtained by the saponification of the corresponding ester.

EXPERIMENTAL

α-p-Nitrocarbobenzoxy-ω-nitro-L-arginine. ω-Nitro-L-arginine (2.19 g., 10 mmoles) was dissolved in 1 *N* NaOH (20 ml., 20 mmoles); the solution was stirred vigorously and cooled to −5° and *p*-nitrocarbobenzoxy chloride[6] (2.16 g., 10 mmoles) dissolved in tetrahydrofuran (15 ml.) was added during 25 minutes in five approximately equal portions The reaction mixture was stirred at room temperature for half an hour. The resulting alkaline solution was washed twice with ethyl acetate and was acidified with 1 *N* HCl (Congo Red). α-*p*-Nitrocarbobenzoxy-ω-nitro-L-arginine precipitated within a few hours. The crude product was re crystallized from methanol-water. Yield, 3.4 g. (85%); m.p. 145–146°, $[\alpha]_D^{23}$ −8.0° (*c*, 1.11 in acetone). For analysis, the product was dried for 12 hours *in vacuo* over P_2O_5 at 78°.

Anal. Calc'd for $C_{14}H_{18}N_6O_8$: C, 42.21; H, 4.55; N, 21.1. Found: C, 42.31; H, 4.75; N, 20.9.

α-p-Nitrocarbobenzoxy-ω-nitro-L-arginyl-β-phenyl-L-serine ethyl ether. α-*p*-Nitrocarbobenzoxy-ω-nitro-L-arginine (0.796 g., 2 mmoles) was dissolved in tetrahydrofuran (10 ml.) previously dried over sodium, tri-*n*-butylamine (0.47 ml., 2 mmoles) was added. The mixture was cooled with ice-salt mixture and ethyl chloroformate (0.19 ml., 2 mmoles) was added; the mixture was stirred for 15 minutes. After this time, a solution of β-phenyl-L-serine ethyl ester hydrochloride (0.491 g., 2 mmoles) and tri-*n*-butylamine (0.47 ml., 2 mmoles) in tetrahydrofuran (10 ml.) was added. The mixture was stirred for 1 hour at room temperature and the solvent was evaporated *in vacuo* at 50–60°. The residue, a thick oil, was dissolved in ethyl acetate (35 ml.) and this solution was washed with 5% aqueous bicarbonate, water, 1 *N* HCl and water, then dried over Drierite. The solvent was evaporated *in vacuo* and the residue was recrystallized from ethanol-water. Yield, 0.77 g., (65%), m.p. 116–117°. For analysis a sample was dried for 18 hours *in vacuo* over P_2O_5 at 78°.

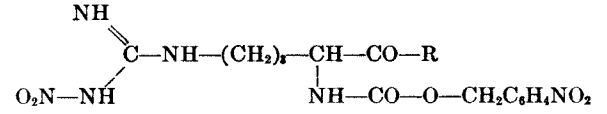
Anal. Calc'd for $C_{25}H_{31}N_7O_{10}$: C, 50.92; H, 5.31; N, 16.63. Found: C, 50.88; H, 5.26; N, 16.65.

α-p-Nitrocarbobenzoxy-ω-nitro-L-arginyl-β-phenyl-L-serine. α-*p*-Nitrocarbobenzoxy-ω-nitro-L-arginyl-β-phenyl-L-serine ethyl ester (0.589 g., 1 mmole) was dissolved in methanol (4 ml.); 1 *N* NaOH (2 ml., 2 mmoles) was added and the mixture was allowed to stand at room temperature for 1 hour. The alkaline solution was washed with ethyl acetate, acidified to Congo Red with 1 *N* HCl; the product crystallized after a few hours; it was recrystallized from methanol-

(6) Prepared according to Gish and Carpenter, *J. Am. Chem. Soc.*, **74**, 3818 (1952).

TABLE I

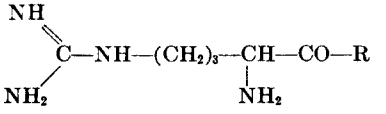
α-*p*-NITROCARBOBENZOXY-ω-NITRO-L-ARGINYL INTERMEDIATES



	Yield, %	M.p., °C.	$[\alpha]_D^{22}$ in acetone	Formula	Analysis Calc'd C	Calc'd H	Calc'd N	Found C	Found H	Found N
—OH	85	145–146	−8.0° (*c*, 1.11)	$C_{14}H_{18}N_6O_8$	42.21	4.55	21.1	42.31	4.75	20.9
—NH—C_6H_5	79	92–93	−1.0° (*c*, 0.78)	$C_{20}H_{22}N_7O_7$	50.73	4.87	20.72	50.53	4.82	20.51
—NH—CH_2—$COOC_2H_5$.H_2O	51	103–107	+7.1° (*c*, 1.15)	$C_{16}H_{25}N_9O_7$.H_2O	43.31	5.42	19.55	43.62	5.15	19.73
—NH—CH($CH_2C_6H_5$)—$COOC_2H_5$	69	165	+8.8° (*c*, 0.65)	$C_{25}H_{31}N_7O_9$	52.33	5.45	17.12	52.64	5.63	17.0
—NH—CH($COOC_2H_5$)—CH_2—CH(CH_3)$_2$	71	154–155	−3.0° (*c*, 1.23)	$C_{22}H_{33}N_7O_9$	49.15	6.16	18.17	49.02	6.28	18.14
—NH—CH($COOC_2H_5$)—CH(OH)—C_6H_5	65	116–117	—	$C_{25}H_{31}N_7O_{10}$	50.92	5.31	16.63	50.88	5.26	16.65
—NH—CH($COOC_2H_5$)—$CH_2C_6H_4OH$	70	139–140	+9.1° (*c*, 2.57)	$C_{25}H_{31}N_7O_{10}$	50.92	5.31	16.63	50.92	5.47	16.60
—NH—CH($COOC_2H_5$)—CH_2—$CH_2COOC_2H_5$	61	Approx. 110	+2.2° (*c*, 1.95)	$C_{23}H_{33}N_7O_{11}$	47.20	5.66	16.75	47.00	5.46	16.55
			$[\alpha]_D^{22}$ in pyridine							
—NH—CH_2—COOH.H_2O	78	128–129	+11.3° (*c*, 1.84)	$C_{16}H_{21}N_7O_9$.H_2O	40.76	4.48	20.8	40.51	4.28	20.42
—NH—CH($CH_2C_6H_5$)—COOH	96	255–256	+14.0° (*c*, 1.53)	$C_{23}H_{27}N_7O_9$	50.63	4.96	17.97	50.23	5.03	17.77
—NH—CH(COOH)—CH_2—CH(CH_3)$_2$	91	193–194	—	$C_{20}H_{29}N_7O_9$	46.92	5.71	19.17	47.07	5.66	19.17
—NH—CH(COOH)—CH_2—C_6H_4OH	98	239–240	+22.4° (*c*, 1.46)	$C_{21}H_{27}N_7O_{10}$	49.18	4.84	17.46	49.0	4.88	17.36
—NH—CH(COOH)—CH(OH)—C_6H_5	90	219–220	+5.3° (*c*, 1.20)	$C_{23}H_{27}N_7O_{10}$	49.18	4.84	17.46	49.0	4.98	17.36
—NH—CH(COOH)—CH_2—CH_2COOH	91	136–137	+6.6° (*c*, 3.24)	$C_{19}H_{25}N_7O_{11}$	43.26	4.77	18.59	43.59	4.99	18.37

TABLE II

L-ARGINYL-DIPEPTIDES



L-Arginyl-R	Yield, %	M.p., °C.	$[\alpha]_D^{23}$ in water	Formula	Analysis					
					Calc'd C	Calc'd H	Calc'd N	Found C	Found H	Found N
—glycine acetate, acetic acid solvate —NH—CH_2—COOH.CH_3COOH, CH_3CO_2H	78	166–167	+47.1° (*c*, 0.89)	$C_{10}H_{21}N_5O_5$, CH_3COOH	41.01	7.20	19.91	41.6	7.51	20.21
L-phenyl-alanine acetate —NH—CH($CH_2C_6H_5$)—COOH.CH_3CO_2H	73	162–163	+30.1° (*c*, 1.84)	$C_{17}H_{27}N_5O_5$	53.5	7.1	18.4	53.2	7.0	18.4
L-leucine acetate —NH—CH(COOH.CH_3CO_2H)—CH_2—CH(CH_3)(CH_3)	85	181–182	+8.9° (*c*, 1.39)	$C_{11}H_{19}N_5O_5$	43.4	8.4	20.2	48.0	8.2	20.4
L-tyrosine acetate —NH—CH(CH_2—C_6H_4OH)—COOH.CH_3CO_2H	79	160–162	+32.0° (*c*, 0.86)	$C_{17}H_{27}N_5O_6$	51.4	6.8	17.6	51.0	6.7	17.5
β-phenyl-L-serine acetate —NH—CH(CH(OH)—C_6H_5)—COOH.CH_3CO_2H	81	168–170	+39.7° (*c*, 0.73)	$C_{17}H_{27}N_5O_6$	51.4	6.8	17.6	51.1	6.7	17.6
L-glutamic acid —NH—CH(COOH)—CH_2—CH_2—COOH	90	202–205	+25.1° (*c*, 18.5)	$C_{11}H_{21}N_5O_5$	43.6	7.0	23.2	43.0	7.0	23.3

water. Yield, 0.490 g. (87%), m.p. 219–220°. $[\alpha]_D^{22}$ +5.3° (*c*, 1.20 in pyridine). For analysis a sample was dried for 12 hours *in vacuo* over P_2O_5 at 78°.

Anal. Calc'd for $C_{23}H_{27}N_7O_{10}$: C, 49.18; H, 4.84; N, 17.46. Found: C, 49.0; H, 4.98; N, 17.36.

L-*Arginyl-β-phenyl-*L-*serine (acetate).* α-*p*-Nitrocarbobenzoxy-ω-nitro-L-arginyl-β-phenyl-L-serine (0.400 g.) was suspended in 95% ethanol (20 ml.) containing 25% of glacial acetic acid. The compound was hydrogenated for 18 hours at room temperature and at atmospheric pressure over 10% palladium on carbon (100 mg.). The catalyst was separated by filtration and the solvent was concentrated *in vacuo* at 30–40° to a volume of 10 ml.; the product then was precipitated with ether. The dipeptide was recrystallized from water-ethanol-ether. Yield: 0.298 g. (91%), m.p. 168–170° $[\alpha]_D^{23}$ +39.7° (*c*, 0.73 in water). For analysis the product was dried for 18 hours *in vacuo* at 78°.

Anal. Calc'd for $C_{17}H_{27}N_5O_6$: C, 51.4; H, 6.8; N, 17.6. Found: C, 51.1; H, 6.7; N, 17.6.

The products and intermediates prepared are shown in Tables I and II.

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF MONTREAL
MONTREAL, CANADA

The Conjugative Ability of Substituent Groups

LEON D. FREEDMAN AND G. O. DOAK

Received March 12, 1956

The effect of substituents on the reactivity of the benzene ring and of side chains attached to it can be quantitatively described by means of the Hammett equation:[1]

$$\log k - \log k^\circ = \rho\sigma$$

The ρ and σ constants are being used by an ever-increasing number of investigators not only for summarizing experimental data but also for making various deductions about the nature of substituents. The present note is concerned with the use of substituent constants for determining the conjugative ability of electron-attracting groups.

Hammett found that in one case, that of the *p*-nitro group, it is impossible to use a single value of σ for all reactions; two distinctly different values are necessary. The larger of these (called σ^* by Jaffé[2]) gives satisfactory results with reactions of aniline and phenol derivatives; the other value (the "normal" σ value) applies to reactions to all other compounds. Table I lists the electron-attracting groups which are now known to require two substituent constants.

This duality of substituent constants has been interpreted as evidence for resonance interaction of the substituent with amino, hydroxy, and similar groups.[3] Furthermore, some authors[4] have assumed that the magnitude of the difference between σ^* and σ may be taken as a measure of the conjugative ability of a substituent. If this assumption is correct, the substituents in Table I should be in order of their conjugative ability. For the most part, the relative order of the conjugative ability does not appear unreasonable. There is, however, a striking anomaly. The difference between σ^* and σ for the *p*-carboxy group places it near the top of the list; the corresponding difference for the *p*-carboethoxy group is near the bottom. It does not seem likely that two such similar groups have such different conjugative abilities.

TABLE I

GROUPS WITH TWO SUBSTITUENT CONSTANTS

Group	$\sigma^* - \sigma$
p-CHO	0.91[a]
p-NO_2	.49[b]
p-COOH	.46[a]
p-CN	.37[a]
p-$COCH_3$	.36[a]
p-SO_2CH_3	.32[c]
p-$S(CH_3)_2^+$	.26[d]
p-$SOCH_3$	.25[e]
p-SO_3^-	.21[f]
p-$Si(C_6H_5)_3$	.20[g]
p-$PO(OC_2H_5)_2$	.19[h]
p-$Ge(C_6H_5)_3$	.16[g]
p-$COOC_2H_5$	.16[c]
p-$Si(CH_3)_3$	.14[i]

[a] The value for σ^* was taken from ref. 1; the value for σ was taken from ref. 2. [b] Taken from ref. 1. [c] Taken from ref. 2. [d] Taken from ref. 4b. [e] Taken from Bordwell and Boutan, *Abstracts of Papers, 124th Meeting, American Chemical Society, Chicago, Ill.*, September, 1953, p. 81-O. [f] Taken from Zollinger, *Nature*, **172**, 257 (1953). [g] Taken from ref. 4c. [h] Taken from Freedman and Jaffé, *J. Am. Chem. Soc.*, **77**, 920 (1955). [i] Taken from ref. 4a.

According to Sklar,[5] the effect of a substituent on the ultraviolet absorption spectrum of benzene depends mainly on the degree of resonance interaction between the substituent and the phenyl radical. Since we have found (*cf.* Table II) that the spectra of benzoic acid and ethyl benzoate are virtually identical, there is probably no appreciable difference between the conjugative effects of the *p*-carboxy and the *p*-carboethoxy groups. Doub and Vandenbelt[6] have concluded that the displacement of the "primary absorption band" of benzene may

(1) Hammett, *Physical Organic Chemistry*, McGraw-Hill Book Co., Inc., New York, N. Y., 1940, Chapter VII.

(2) Jaffé, *Chem. Revs.*, **53**, 191 (1953).

(3) (a) Branch and Calvin, *The Theory of Organic Chemistry*, Prentice-Hall, Inc., New York, N. Y., 1941, pp. 257, 417; (b) Bordwell and Cooper, *J. Am. Chem. Soc.*, **74**, 1058 (1952); (c) Bordwell and Anderson, *J. Am. Chem. Soc.*, **75**, 6019 (1953).

(4) (a) Benkeser and Krysiak, *J. Am. Chem. Soc.*, **75**, 2421 (1953); (b) Bordwell and Boutan, *J. Am. Chem. Soc.*, **78**, 87 (1956); (c) Benkeser, DeBoer, Robinson, and Sauve, *J. Am. Chem. Soc.*, **78**, 682 (1956).

(5) Sklar, *J. Chem. Phys.*, **7**, 984 (1939).

(6) Doub and Vandenbelt, *J. Am. Chem. Soc.*, **69**, 2714 (1947).