(lit.[26] m.p. 181–182°); infrared spectrum (KBr), OH 2.85-(m) μ and C=O 5.75(s) μ.

Acidification of the combined base washings, extraction with ether, drying, and solvent evaporation led to 50 mg. of non-crystalline acidic material, $[\alpha]_D$ −38° (EtOH); ultraviolet spectrum (95% ethanol), λ_{max} 241 mμ. The acidic substance was dissolved in 0.1 ml. of acetone, 2 drops of di-*n*-amylamine (b.p. 190–192°) added[37] and the solution cooled. Filtration of the crystalline precipitate and crystallization from acetone furnished a salt, $[\alpha]_D$ −53° (EtOH), whose infrared spectrum was identical with that of a freshly prepared sample of the di-*n*-amylamine salt of abietic acid, $[\alpha]_D$ −59° (EtOH).

Identical acid treatment and work-up of 100 mg. of isopimaric acid yielded 17 mg. of a non-crystalline mixture of 5- and 6-lactones, 26 mg. of hydroxylactone, m.p. 160–175°, increased to 180–181° after crystallization from petroleum ether–acetone, no depression on admixture with above hydroxylactone, identical infrared spectra, and 45 mg. of acid, $[\alpha]_D$ −45° (EtOH), ultraviolet spectrum (95% ethanol), λ_{max} 241 mμ, whose di-*n*-amylamine salt, $[\alpha]_D$ −58° (EtOH), had an infrared spectrum identical with that of the abietic acid salt.

(37) *Cf.* G. C. Harris and T. F. Sanderson, THIS JOURNAL, **70**, 334 (1948).

AMES, IOWA

[CONTRIBUTION NO. 500 FROM THE CENTRAL RESEARCH DEPARTMENT, EXPERIMENTAL STATION, E. I. DU PONT DE NEMOURS AND CO.]

Aminodihydrofuramides from 3-Amino-1-propynes and Carbon Monoxide

BY J. C. SAUER, B. W. HOWK AND R. T. STIEHL

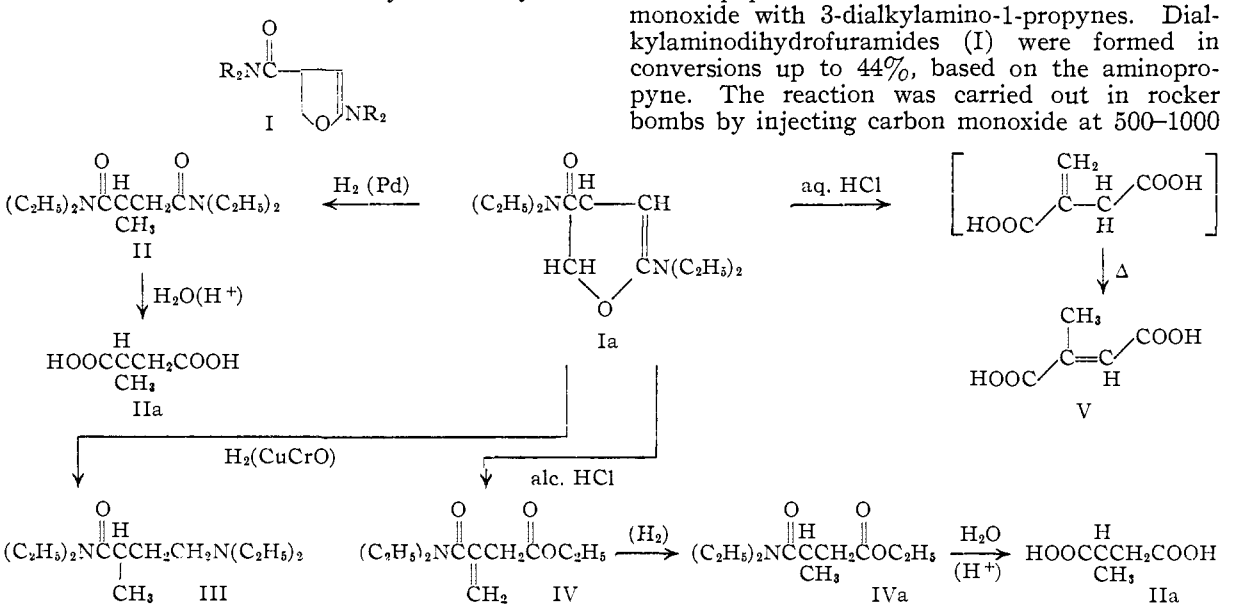
RECEIVED JULY 28, 1958

The reaction of 3-diethylamino-1-propyne with carbon monoxide has given N,N-diethyl-5-diethylamino-2,3-dihydro-3-furamide in 44% yield. The synthesis was carried out by injecting carbon monoxide at 500–1000 atm. into the aminopropyne dissolved in a ketone solvent at a temperature of 125°. Catalytic amounts of dicobalt octacarbonyl were necessary for the synthesis. The reaction has also been extended to several other aminopropynes. The products undergo facile ring cleavage with hydrogen, hydrogen chloride or water to give succinic acid derivatives. The mechanism for this unusual transformation is unknown, in part because other products of the reaction were isolable only as intractable residues.

The literature contains many references to the reactions of acetylene or substituted acetylenes with carbon monoxide. These reactions generally involved various metallic carbonyls as catalysts or reactants, and the products were mainly acrylic compounds or hydroquinones.[1] Although a number of functionally substituted acetylenes have been studied in these reactions, only one reference to the interaction of 3-dialkylamino-1-propynes with carbon monoxide appears to have been reported. With iron carbonyl hydride furnishing the carbon monoxide needed for the synthesis, several 3-dialkylamino-1-propynes were converted into the 2,5-bis-(dialkylaminomethyl)-hydroquinones.[2]

This paper describes a new reaction of carbon monoxide with 3-dialkylamino-1-propynes. Dialkylaminodihydrofuramides (I) were formed in conversions up to 44%, based on the aminopropyne. The reaction was carried out in rocker bombs by injecting carbon monoxide at 500–1000 atm. into a solution of the aminopropyne at 125°. Ketones such as acetone or cyclohexanone were the best solvents tested. Catalytic amounts of dicobalt octacarbonyl were necessary for the synthesis.

The novel products were identified mainly on the basis of the chemical evidence indicated schematically below. N,N-Diethyl-5-diethylamino-2,3-dihydro-3-furamide (Ia) undergoes facile ring cleavage at the bond in the 1,2-position. Open-chain compounds, all of which may be considered to be derivable from methylsuccinic acid, were formed in

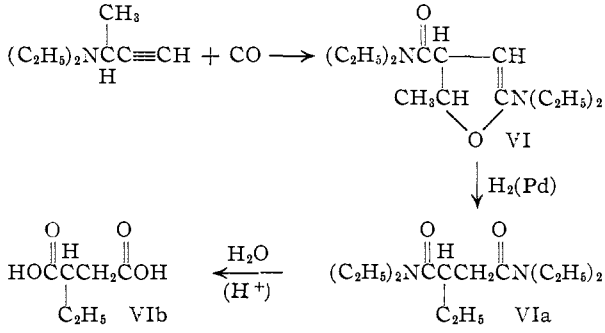


(1) (a) J. W. Copenhaver and M. H. Bigelow, "Acetylene and Carbon Monoxide Chemistry," Reinhold Publishing Corp., New York, N. Y., 1949; (b) J. W. Reppe, *et al.*, *Ann.*, **582**, 1 (1953); (c) E. R. H. Jones, T. Y. Shen and M. C. Whiting, *J. Chem. Soc.*, 230 (1950); 48, 763, 766 (1951); (d) E. R. H. Jones, G. H. Whitham and M. C. Whiting, *ibid.*, 1865 (1954).

(2) Reference 1a, p. 293; J. W. Reppe, *et al.*, *Ann.*, **582**, 142 (1953).

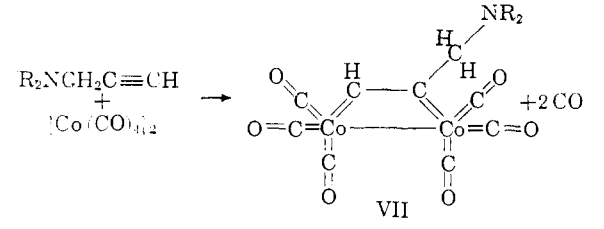
good yields by reaction with hydrogen, alcohol or water.

In addition to Ia, similar dihydrofuramides were prepared from 3-dimethylamino-1-propyne, 3-(4-morpholino)-1-propyne and 3-diethylamino-3-methyl-1-propyne. This latter compound is of particular interest in that the carbon atom attached to the nitrogen of the aminoacetylene was "tagged" with a methyl group. This "tagged" methyl group was shown to be in the 2-position of the furan ring, as indicated schematically.

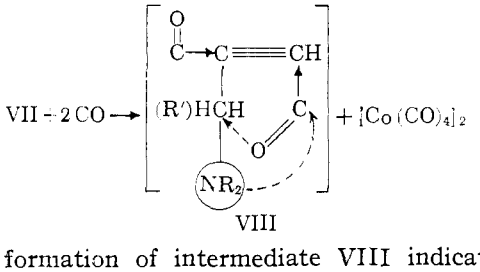


Compound VI was characterized by hydrogenation to the bis-N,N-diethylamide of ethylsuccinic acid (VIa). Hydrolysis of VIa gave ethylsuccinic acid (VIb).

Although the mechanism for this unusual transformation is unknown, the following observation is of interest. It was noted that the dicobalt octacarbonyl catalyst reacted with the aminopropyne releasing carbon monoxide, probably producing VII as the initial intermediate in the synthesis.[3] It is



postulated that carbon monoxide reacted with VII giving an intermediate along the lines suggested by VIII.



The formation of intermediate VIII indicates a catalytic role for the dicobalt octacarbonyl and suggests a precursor for a dihydrofuramide ring with substituents in the 3- and 5-positions.

Significant amounts of intractable solids or oils were always present as by-products of the reaction. No clue as to the nature of this residue, or its precursor, has been obtained. An elemental analysis of such a residue from a synthesis of Ia indicated an approximate empirical formula of $C_{15}H_{20}NO_4$.

(3) See H. W. Sternberg, *et al.*, THIS JOURNAL, **76**, 1457 (1954).

Experimental

N,N-Diethyl-5-diethylamino-2,3-dihydro-3-furamide (Ia).—A 500-ml. stainless-steel rocker bomb was swept out with oxygen-free nitrogen and was then charged with 150 ml. of acetone, 1 g. of dicobalt octacarbonyl and 25 g. of 3-diethylamino-1-propyne. The bomb was closed, cooled in a mixture of solid carbon dioxide–methanol, and evacuated to about 5 mm. The bomb was installed behind a heavy barricade and connected to a source of carbon monoxide. All subsequent operations were controlled from the outside. The reaction mixture was heated to 125° for approximately 16 hours during which the gage pressure was maintained at 600–1000 atm. by periodic injection of carbon monoxide. The bomb was next cooled to room temperature, pressure released, and the contents removed. The reaction mixture was poured into hexane (500 ml.) and filtered to remove about 20 g. of a dark brown solid. Evaporation of the filtrate on a steam-bath left a thick oil. Fractionation of this oil gave N,N-diethyl-5-diethylamino-2,3-dihydro-3-furamide (Ia), b.p. 124–126° (0.28 mm.), $n^{25}D$ 1.4930, d^{25}_4 0.9962 (11.9 g., 44% yield). Smaller amounts of intractable residues were found when ketones were used as solvents than when acetonitrile or benzene were employed.

Characterization of N,N-Diethyl-5-diethylamino-2,3-dihydro-3-furamide (Ia). Elemental, Functional Group and Spectral Analyses of Ia.—*Anal.* Calcd. for $C_{13}H_{24}O_2N_2$: C, 65.0; H, 10.0; N, 11.6; g. H_2/g., 0.0083; mol. wt., 240. Found: C, 64.9; H, 10.1; N, 11.5; g. H_2/g., 0.0084; mol. wt., 240. A saponification number of zero was obtained under the usual conditions for this determination. Saponification of an authentic model compound, N,N-diethylacetamide, was also zero under these conditions. The infrared spectrum indicated amide carbonyl absorption at 6.15 μ. A shoulder at 6.05 μ was ascribed to the unconjugated carbon–carbon double bond of the type present in Ia. The ultraviolet spectrum showed a weak maximum at 2400 A. (k 25). The molecular refractivity value was 70.1 compared to a theoretical value of 69.4. The proton magnetic resonance spectrum for Ia gave

	=CH	$-CH_2-$	Ring CH	$-CH_3$
δ	+0.12	−0.15	−0.29	−0.38
Rel. intensities	1	8.4	3.2	12.4
"Theor." value	1	8	3	12

Characterization of N,N-Diethyl-5-diethylamino-2,3-dihydro-3-furamide (Ia) Through Derivatives. Catalytic Hydrogenation of Ia to Bis-N,N-diethylamide of Methylsuccinic Acid (II).—Hydrogenation of Ia (36 g.) in dioxane containing palladium-on-carbon catalyst gave 32.5 g. of II, b.p. 130–131.5° (2 mm.), $n^{25}D$ 1.4687 (32.5 g., 90%) yield). The hydrogenation was carried out in a shaker tube at 2000 lb./sq. in. pressure and 60° for 3 hours. *Anal.* Calcd. for $C_{13}H_{26}O_2N_2$: C, 64.5; H, 10.7; N, 11.5. Found: C, 64.2; H, 10.6; N, 11.4. The infrared spectrum, identical to that of an authentic sample, showed absorption at 6.1 μ for amide carbonyl and absorption at 3.35 and 3.4 μ for saturated CH. The authentic sample of II was prepared from the acid chloride of methylsuccinic acid[4] and diethylamine.

Sodium Reduction of Ia to Bis-N,N-diethylamide of Methylsuccinic Acid (II).—Compoud Ia (15 g., 0.06 mole) was reduced by sodium (25.9 g., 1.1 g. atoms) in refluxing absolute ethyl alcohol; II was isolated by ether extraction of the reaction mixture previously acidified with dilute acetic acid. There was obtained 8 g. (53% yield) of II, distilling at 118–120° (1.1 mm.), $n^{25}D$ 1.4688.

Hydrolysis of Bis-N,N-diethylamide of Methylsuccinic Acid (II).—Compound II (2.0 g.) was refluxed for 8 hours with 25 ml. of concentrated hydrochloric acid, evaporated to dryness, made alkaline, and evaporated to dryness again. The mixture was again acidified and evaporated under a pressure of 20–40 mm. until nearly dry. The residue was quickly extracted with refluxing absolute alcohol. The methylsuccinic acid, after quickly removing the alcohol, was recrystallized from an ethyl acetate–petroleum ether mixture, m.p. 104–106°. A mixed melting point with an authentic sample showed no depression.

Hydrogenation of Ia to N,N-Diethylamide of α-Methyl-γ-diethylaminobutyric Acid (III).—Hydrogenation of Ia (24 g.) with copper chromite catalyst in dioxane solvent gave 8.5 g. (37%) of an aminoamide distilling at 112–115° (2 mm.),

(4) G. F. Morrell, *J. Chem. Soc.*, **113**, 1736, 2706 (1914).

$n^{25}D$ 1.4548, having the probable structure III. The hydrogenation was carried out at 260° and 3000 lb./sq. in. pressure during 2 hours. *Anal.* Calcd. for $C_{13}H_{28}ON_2$: C, 68.3; H, 12.2; N, 12.2; neut. equiv., 228. Found: C, 68.5; H, 12.3; N, 12.0; neut. equiv., 229. The infrared spectrum showed amide carbonyl absorption at 6.1 μ, saturated CH absorption at 3.4 μ, and weak absorption at 3.6 μ indicative of $\begin{matrix} C \\ \diagdown \\ C \end{matrix}\!\!>\!N\text{-}CH_2\text{-}$. The spectrum was very similar but not identical to that of a "model" compound, $(C_2H_5)_2NC(=O)(CH_2)_4N(C_2H_5)_2$. This "model" compound was prepared by adding diethylamine to δ-chlorovaleroyl chloride. The "model" compound, δ-diethylamino-N,N-diethylvaleramide, distilled at 103° (1 mm.), $n^{25}D$ 1.4593. *Anal.* Calcd. for $C_{13}H_{28}ON_2$: C, 68.3; H, 12.2; N, 12.2. Found: C, 68.1; H, 12.1; N, 12.0.

Preparation of Amidoester of Itaconic Acid (IV) from Ia.—In 150 ml. of anhydrous ether was placed Ia (21 g.) and the solution saturated with dry hydrogen chloride. After standing a few minutes, anhydrous ethanol (200 ml.) was added, and all of the ether and part of the alcohol were removed at a bath temperature of 20–60° (20–40 mm.). Additional anhydrous ether was added to the residual mush, and the diethylamine hydrochloride was removed by filtration (8.9 g., 93%). After removing the last of the ether and ethanol, the filtrate distilled almost entirely at 124–127° (5 mm.), $n^{25}D$ 1.4731 (12.1 g., 65% yield). *Anal.* Calcd. for $C_{11}H_{19}O_3N$: C, 62.0; H, 9.0; N, 6.6; g. H_2/g., 0.0094; sap. no., 264. Found: C, 62.1; H, 8.9; N, 6.6; g. H_2/g., 0.0098; sap. no., 269. The infrared spectrum showed absorption at 6.15, 5.8 and 3.4 μ for amide >C=O, ester >C=O and saturated CH, respectively. Weak absorption at 11.3 μ was interpreted as indicating the $CH_2{=}C\begin{matrix} C \\ \diagup\!\diagdown \\ C \end{matrix}$ function.

Further proof for the structure of IV consisted in hydrogenation followed by hydrolysis to methylsuccinic acid; IV (9 g.) was hydrogenated in dioxane solvent using palladium-on-carbon catalyst at 65° and 1500 lb./sq. in pressure during 2 hours. The resulting amidoester of methylsuccinic acid (IVa) distilled at 82–87° (2 mm.), 7.5 g. (84%), $n^{25}D$ 1.4508. *Anal.* Calcd. for $C_{11}H_{21}O_3N$: C, 61.4; H, 9.8; N, 6.5. Found: C, 62.0; H, 9.8; N, 6.1. The infrared spectrum showed absorption at 6.1, 5.8 and 3.4 μ for amide >C=O, ester >C=O and saturated CH, respectively.

Compound IVa (1.5 g.) was hydrolyzed in concentrated hydrochloric acid using the procedure described above for the hydrolysis of II. After recrystallization from an ethyl acetate–petroleum ether solvent, methylsuccinic acid (IIa) melted at 103–104°. A mixed melting point with an authentic sample of methylsuccinic acid showed no depression. *Anal.* Calcd. for $C_5H_8O_4$ (IIa): C, 45.4; H, 6.1. Found: C, 45.7; H, 6.3. The *p*-bromophenacyl esters of IIa and the authentic sample each melted at 114–115°, and the mixed melting point showed no depression. The infrared spectra of these esters were identical, showing absorption at 3.25, 3.45, 5.8, 5.9, 6.3, 7.3 and 12.3 μ for aromatic CH, saturated CH, ester >C=O, conjugated ketone >C=O, aromatic >C=C<, $-CH_3$ and *p*-disubstituted aromatic group, respectively.

Preparation of Mesaconic Acid (V) from Ia.—Compound Ia (7 g.) was refluxed with concentrated hydrochloric acid (45 ml.) for 16 hours. The mixture was evaporated on a steam-bath. The concentrate was made alkaline with caustic and again evaporated to dryness to remove diethylamine. The resulting mixture was acidified with dilute hydrochloric acid and concentrated at 60–80° (20–30 mm.). The resulting residue was extracted with ethanol, and ether was added to this extract to the cloud point. Upon cooling, a crop of crystals melting at 197–198° was obtained. It is believed that the intermediate in this hydrolysis was itaconic acid, which is known to isomerize to mesaconic acid (V) under the conditions employed.[5] *Anal.* Calcd. for $C_5H_6O_4$ (V): C, 46.2; H, 4.6; neut. equiv., 65.0. Found: C, 46.3; H, 4.9; neut. equiv., 66.0. The infrared patterns of V and an authentic sample of mesaconic acid were identical, both spectra showing absorption at 5.9 and 6.05 μ for acid >C=O and >C=C,< respectively. Broad absorption at 3–4 μ indicated CH and carboxylic –OH.

(5) R. Fittig and A. Landott, *Ann.*, **188**, 73 (1877).

Analysis of Intractable By-product.—Found: C, 64.2; H, 6.9; N, 5.2. This residue also contained a small amount of cobalt. An approximate empirical formula is $C_{15}H_{20}NO_4$.

N,N-Diethyl-2-methyl-5-diethylamino-2,3-dihydro-3-furamide (VI) was made from 3-diethylamino-1-butyne and carbon monoxide using the procedure described above. From 150 g. of the aminopropyne there was obtained 45 g. (29%) of VI distilling at 120° (2 mm.), $n^{25}D$ 1.4876. *Anal.* Calcd. for $C_{14}H_{26}O_2N$: C, 66.3; H, 10.3; N, 11.0; g. H_2/g., 0.0071. Found: C, 66.2; H, 10.3; N, 10.4; g. H_2/g., 0.0077. The infrared spectrum was similar to that for Ia and showed amide >C=O absorption at 6.1 μ The proton magnetic resonance spectrum was consistent with the structure for VI.

Compound VI (16 g.) was hydrogenated in dioxane with palladium-on-carbon catalyst to give the bis-amide of ethylsuccinic acid (VIa), b.p. 128–130° (3 mm.), $n^{25}D$ 1.4743 (9 g., 56%). The hydrogenation was carried out at 60° and 2000 lb./sq. in. during 2 hours. *Anal.* Calcd. for $C_{14}H_{28}O_2N_2$: C, 65.6; H, 10.9; N, 10.9. Found: C, 65.5; H, 10.6; N, 10.2. The bis-amide (VIa) was hydrolyzed by refluxing in concentrated hydrochloric acid to give ethylsuccinic acid (VIb), m.p. 96–98°. *Anal.* Calcd. for $C_6H_{10}O_4$: C, 49.3; H, 6.9; neut. equiv., 72.0. Found: C, 49.7; H, 6.3; neut. equiv., 72.5. The *p*-bromophenacyl esters of VIb and authentic ethylsuccinic acid each melted at 97–99°, and a mixed melting point showed no depression. The infrared spectra of the *p*-bromophenacyl esters were identical, showing absorption at 3.25, 3.35 and 3.4 μ; 5.75, 5.85, 6.27 and 6.7 μ; and 12.25 μ for aromatic CH, saturated CH, ester >C=O, conjugated >C=O, aromatic >C=C<, and *p*-disubstituted aromatic groups, respectively. *Anal.* of the *p*-bromophenacyl ester of ethylsuccinic acid (VIb) Calcd. for $C_{22}H_{20}O_6Br_2$: C, 49.1; H, 3.7. Found: C, 49.4; H, 3.8. The sample of "authentic"ethylsuccinic acid was made by the method of Smith.[6]

N,N-Dimethyl-5-dimethylamino-2,3-dihydro-3-furamide was made from 3-dimethylamino-1-propyne and carbon monoxide in 18% conversion in the manner previously described. The product distilled at 125–127° (5 mm.) and melted at 71–73° after a recrystallization from an ethyl acetate–petroleum ether mixture. *Anal.* Calcd. for $C_9H_{16}O_2N_2$: C, 58.7; H, 8.7; H, 15.2. Found: C, 58.1; H, 8.7; N, 15.1. The infrared spectrum was similar to that for Ia and showed absorption at 6.0, 6.1 and 6.17 μ for amide >C=O. The proton magnetic resonance spectrum was consistent with the assigned structure.

Morpholine amide of 5-(4-morpholino)-2,3-dihydro-3-furoic acid was prepared from 3-(4-morpholino)-amino-1-propyne and carbon monoxide by the procedure described above. The crude amide was precipitated from the reaction mixture with ether and was distilled at 182–192° (0.6 mm.) (8.0 g., 24%). It was identified on the basis of the infrared spectrum and analytical data. *Anal.* Calcd. for $C_{13}H_{20}N_2O_4$: C, 58.2; H, 7.5; N, 10.4; mol. wt., 268. Found: C, 58.8; H, 7.7; N, 10.1; mol. wt., 246. The infrared spectrum showed absorption at 6.1 μ for amide >C=O, and absorption at 9.0 μ and 11.8 μ for the morpholino group. The proton magnetic resonance spectrum was consistent with the assigned structure.

3-Diethylamino-1-propyne.—Diethylamine (110 g., 1.5 moles) was added to rapidly stirred 37% aqueous formaldehyde (123 g., 1.5 moles) at such a rate that the temperature was maintained at 30–35°, with ice cooling when necessary. The resultant two-phase mixture was then combined with 10 g. of cuprous chloride (commercial grade) in a 500-ml. rocker bomb. The bomb was cooled in a solid carbon dioxide–acetone-bath and alternately evacuated and flushed with nitrogen several times. The bomb was then connected to a source of acetylene. The reaction mixture was slowly heated to 80° where it was maintained for a period of 9 hours during which the gage pressure was maintained at 220 lb./sq. in. Repressuring with acetylene was necessary, and approximately 1.8 moles of acetylene was consumed. The bomb was then cooled to room temperature and vented. The reaction mixture was filtered, and the two-phase filtrate was gently distilled on a heated water-bath under water aspirator pressure. The two phases of the distillate were separated, and the bottom layer was saturated with solid potassium carbonate and then extracted twice with 200-ml. por-

(6) P. A. S. Smith and J. P. Horwitz, THIS JOURNAL, **71**, 3418 (1949).

tions of ether. The ether extracts and the top layer were then combined and dried over potassium carbonate. Ether was removed on a steam-bath, and 126 g. of 3-diethylamino-1-propyne, distilling at 119–120°, was obtained (76% yield, $n^{25}D$ 1.4288). This procedure has obvious advantages over the literature method which requires diethylamine acetate.[7]

3-(4-Morpholino)-1-propyne.—3-(4-Morpholino)-1-propyne, b.p. 89–92° (38 mm.) $n^{25}D$ 1.4723, was obtained in 66% yield by utilizing the procedure described above. This propyne was identified by infrared and nuclear magnetic resonance spectra and analytical data. *Anal.* Calcd. for $C_7H_{11}NO$: C, 67.2; H, 8.9; N, 11.2; mol. wt., 125. Found: C, 67.7; H, 8.9; N, 10.9; mol. wt., 123, 128. The infrared spectrum indicated ≡CH (3.0 μ), saturated CH (3.4, 3.5 and 3.7 μ), and —C≡C— (4.75 μ). The nuclear magnetic resonance spectrum indicated acetylenic hydrogen and two types of methylene hydrogen.

3-Diethylamino-3-methyl-1-propyne, b.p. 126–128°, $n^{25}D$ 1.4273, was prepared in 33% yield from acetylene and diethylamine by a published procedure.[8]

(7) Ref. 1a, p. 110; also, Reppe, *Ann.*, **596**, 1 (1955).

(8) C. Gardner, V. Kerrigan, J. D. Rose and B. C. L. Weedon, *J. Chem. Soc.*, 780 (1949).

Reaction of 3-Diethylamino-1-propyne with Dicobalt Octacarbonyl.—3-Diethylamino-1-propyne (0.19 g.) in acetone (25 ml.) was added to dicobalt octacarbonyl (0.57 g.) in the standard Orsat apparatus for measuring gases. There was collected 63.3 ml. of carbon monoxide, making the necessary corrections for acetone vapor (87% of theory).

Commercial-grade acetylene was purified according to a previously described procedure.[9] The infrared spectra were determined on a Perkin–Elmer 21 double-beam spectrometer. The ultraviolet spectra were determined on a Cary model 11 spectrophotometer. The proton magnetic resonance spectra were obtained using a Varian high-resolution n.m.r. spectrometer and electromagnet at frequencies of 40 Mc. and fields of 10,000 gauss, respectively. The spectra were calibrated in terms of displacements in cycles per second (c.p.s.) from the proton resonance of water. Positive values are on the low field side of water, and negative values are on the high field side. Calibration was accomplished by superimposing an audiofrequency on the sweep field to produce side band peaks to the water resonance. Yields of the dihydrofuramides were calculated on the basis of the amount of dialkylamino-propyne consumed.

(9) J. C. Sauer, THIS JOURNAL, **79**, 5314 (1957).

WILMINGTON 98, DEL.

[CONTRIBUTION FROM THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH]

Characterization Studies with Subtilin

BY ALFRED STRACHER[1] AND LYMAN C. CRAIG

RECEIVED JULY 2, 1958

Further purification studies of subtilin A by countercurrent distribution (c.c.d.) are reported. Molecular weight determination by the method of partial substitution has conclusively shown that subtilin A approximates 3300 in molecular weight. This and quantitative amino acid analyses are consistent with an amino acid formula of Asp,Pro,Gly_2,Ala,Val,Ileu,Leu_4,-Phe,Lys_3,Lan,β-MeLan$_4$,Glu_3,Try,Sar_2. Sarcosine, not heretofore reported, has been shown to be an N-terminal group. Subtilin A is a pentacyclic peptide with a side chain.

A number of different polypeptide antibiotics[2] in the molecular weight range up to 1500 have now been well characterized chemically and reasonably certain cyclic structures have been proposed for them. Although larger ones are known they have not thus far been studied as carefully. Subtilin[3] produced by a particular strain of *Bacillus subtilis* is the best characterized and perhaps the most readily available member of the larger size group.

A preliminary survey of the excellent chemical work already done with subtilin[4–9] indicated that it probably was sufficiently well characterized for further structural study. Nonetheless, because of the effort required in such an undertaking it seemed wise to repeat part of the work using different methods. This paper will report studies of this nature and some new observations bearing on the structure of the peptide.

Experimental

Materials.—Three samples of subtilin all received from the Western Regional Laboratory have been studied thus far. One was a 5-g. sample (Lot 317) received from Dr. Harold Olcott in 1950. Two more samples were received from Dr. Alderton in 1957. One of these, 152F, was similar to 317 but freshly prepared whereas 317 had been stored for about 10 years. The other was relatively pure subtilin A obtained by silica gel partition chromatography of 152F. Dr. Alderton had found it to give a single band by countercurrent distribution at 180 transfers (system = 20% acetic acid, 5, *n*-butanol, 4) with close agreement to a calculated curve. It behaved the same way in our hands.

Fractionation Studies.—Fractionation was accomplished by countercurrent distribution in a 1000 tube (2 ml. lower phase) automatic train of the type previously described[10] in the Alderton system. The charge was 1 g. of sample 152F initially scattered in the first twenty tubes of the train. Each transfer required 2.5 minutes including 5 strokes for equilibration and 1.5 minutes for separation of the phases. 5 strokes seemed to be sufficient for equilibration. The temperature of the train was 25°.

After 1000 transfers the upper patterns of Fig. 1 were obtained by optical density measurement at 288 mμ of the upper and lower phases in a Beckman quartz spectrophotometer. Several small bands as indicated and a larger one on the right, C_3, were removed from the train. After these tubes were filled with fresh phases the distribution was continued to 2540 transfers by the recycling procedure.

Analysis by optical density now gave the lower patterns of Fig. 1. A plot of the partition ratio across the main band is given above the distribution curve.

Cuts labeled A_3 and B_3 were taken as indicated on the chart. They were concentrated in a rotary evaporator until all the butanol phase was removed and lyophilized from the aqueous solution. The preparation from cut A_3 was the material used for the characterization studies reported in this paper. It corresponds to subtilin A.

Anal. The sample (from the run of Fig. 1) lost 18.2% on drying at 100° *in vacuo*. Found: C, 52.97; H, 6.99; N, 16.06; S, 4.76; N-CH_3, 1.92.

(1) Fellow of the National Foundation for Infantile Paralysis.

(2) L. C. Craig, Proc. 3rd Int. Cong. Biochem., Brussels, 1955.

(3) K. P. Dimick, G. Alderton, J. C. Lewis, H. D. Lightbody and H. L. Fevold, *Arch. Biochem.*, **15**, 1 (1947).

(4) J. C. Lewis and N. S. Snell, THIS JOURNAL, **73**, 4812 (1951).

(5) N. G. Brink, J. Mayfield and K. Folkers, *ibid.*, **73**, 330 (1951).

(6) G. Alderton and H. L. Fevold, *ibid.*, **73**, 463 (1951).

(7) J. F. Carson, *ibid.*, **74**, 1480 (1952).

(8) G. Alderton, *ibid.*, **75**, 2391 (1953).

(9) H. L. Fevold, K. P. Dimick and A. A. Klose, *Arch. Biochem.*, **18**, 27 (1948).

(10) L. C. Craig, W. Hausmann, E. H. Ahrens, Jr., and E. F. Harfenist, *Anal. Chem.*, **23**, 1236 (1951).