product is identical with the substance resulting from nitration of triphenylethylene itself.

(b) Without solvent: The carbinol V (15 g.) was added in small portions to fuming nitric acid (75 cc.) with stirring. A violent reaction took place, which raised the temperature to 70°. The reaction was completed by heating the mixture to 80° for one and one-half hours. Upon standing for several days, the reaction product crystallized out. It was filtered off, washed with nitric acid, then with water and dried; crude yield, 20 g., 83%. The tetranitro derivative (VII?) crystallized from benzene or butyl acetate in yellow prisms, m. p. 205°.

Anal. Calcd. for $C_{20}H_{12}O_8N_4$: C, 55.0; H, 2.8; N, 12.8. Found: C, 55.4; H, 2.6; N, 12.5.

The same product was obtained, but in a less satisfactory form, by nitration of triphenylethylene or α-nitrotriphenylethylene (VI) with fuming nitric acid.

Summary

The coupling of β,β-diarylacrylic acids with diazotized anilines opens a new route to substituted triarylethylenes. Geometrical isomers of the acids yielded identical coupling products.

Under the experimental conditions of this reaction, α-phenylcinnamic acid is decarboxylated to *trans*-stilbene. Decarboxylation is also a side-reaction for β,β-diarylacrylic acids.

1,1-Diphenyl-2-(*p*-nitrophenyl)-ethylene can be reduced catalytically stepwise first to the unsaturated, then to the saturated, amine. Nitration of triphenylethylene, the corresponding carbinol or its α-nitro derivative with fuming nitric acid in the absence of a solvent produces a tetranitro derivative.

RECHOVOT, PALESTINE RECEIVED AUGUST 25, 1947

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, CORNELL UNIVERSITY MEDICAL COLLEGE]

Reactions of Mustard-type Vesicants with α-Amino Acids[1]

BY VINCENT DU VIGNEAUD, CARL M. STEVENS,[2] HAROLD F. MCDUFFIE, JR.,[3] JOHN L. WOOD[4] AND HERBERT MCKENNIS, JR.[5]

Early in World War II it was considered that reactions between mustard gas (H) and certain enzymes possibly played a role in the mechanism of vesication by H-type compounds. As part of a collaborative effort to uncover the mechanism of vesication by chemical warfare agents, reactions between H-type compounds and proteins were studied in a number of laboratories. Prior to our investigations, published work[6] and available unpublished British reports[7] indicated that the properties of several proteins were altered by treatment with mustard gas. To gain insight into the protein-H reactions, this Laboratory and others investigated the preparation and nature of compounds formed by the reaction of H-type vesicants with amino acids. This report covers part of this one aspect of the larger problem.

From the chemical standpoint, studies on mustard gas are complicated by the fact that H contains two reactive halogens. Since it was already known[8] that several compounds of the type $RSCH_2CH_2Cl$ possess vesicant action, we sought to simplify the problem by employing these "one-handed" vesicants in our chemical studies. Although these compounds containing only one β-chloroethyl group are potent vesicants, they are quantitatively much less vesicant than H itself. Qualitatively their physiological action parallels that of H. The "one-handed" agents, therefore, must be capable of entering into the chemical reactions essential to vesication. The fact that the structures and properties of the molecules closely resemble those of H itself makes it highly probable that the mechanism of vesication is essentially the same in each case. These considerations caused us to focus our attention largely on the one-handed vesicants, which for convenience are designated as follows:

$C_6H_5CH_2SCH_2CH_2Cl$	Benzyl-H
$CH_3SCH_2CH_2Cl$	Methyl-H
$CH_3CH_2SCH_2CH_2Cl$	Ethyl-H
$CH_3CH_2CH_2CH_2SCH_2CH_2Cl$	Butyl-H

A survey of the general literature[9] indicated that H-type vesicants reacted readily with sulfhydryl, amino and phenolic hydroxyl groups in alkaline solution. Furthermore, a derivative of an α-amino acid had been reported. It was the product formed by the reaction[10] of H and glycine ethyl ester, having the structure I.[11]

$$S(CH_2CH_2NHCH_2COOC_2H_5)_2 \qquad I$$

In our experiments we studied the reactions of most of the naturally occurring amino acids with vesciants of the type $RSCH_2CH_2Cl$, where R has the structures indicated above.

(1) The work described in this paper was carried out under Contract OEMsr-144 between the Office of Scientific Research and Development and Cornell University Medical College and is described in Progress Reports to the National Defense Research Committee, January, 1942, to October, 1943.

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(6) Berenblum and Wormall, *Biochem. J.*, **33**, 75 (1939).

(7) Berenblum (1940); Pirie (1941); Peters (1941).

(8) See, for instance, Kirner, THIS JOURNAL, **55**, 3501 (1933); Patterson and du Vigneaud, *J. Biol. Chem.*, **111**, 393 (1935).

(9) See Jackson, *Chem. Rev.*, **15**, 425 (1934).

(10) For recently published observations on this reaction as well as other studies on the reaction of amino acids with H and related compounds, see Boursnell, Francis and Wormall, *Biochem. J.*, **40**, 737 (1946).

(11) Cashmore and McCombie, *J. Chem. Soc.*, 2884 (1923).

TABLE I

Nα-SUBSTITUTED DERIVATIVES OF AMINO ACIDS WITH MUSTARD-TYPE VESICANTS

Compound	Proc. for prepn.	Solvent for recryst.	Approx. yield, %	M. p., °C.	Molecular formula	Analyses, % Calcd. C	Calcd. H	Found C	Found H
N-β-Benzylmercaptoethylglycine[a]	C	Abs. ethanol	40[b]	187–188	$C_{11}H_{15}O_2NS$	58.6	6.71	58.6	6.91
N-β-Benzylmercaptoethyl-DL-alanine[c]	C	Water	50[b]	210–215	$C_{12}H_{17}O_2NS$	60.2	7.16	60.4	7.36
N-β-Benzylmercaptoethyl-DL-valine	B	95% ethanol	40[d]	236–239	$C_{14}H_{21}O_2NS$	62.9	7.91	63.0	7.85
N-β-Benzylmercaptoethyl-L-leucine	A	50% acetic acid	20[d]	229–231	$C_{15}H_{23}O_2NS$	64.0	8.24	64.0	8.13
N-β-Benzylmercaptoethyl-DL-leucine	B	50% ethanol	25[d]	225–228	$C_{15}H_{23}O_2NS$	64.0	8.24	64.1	7.62
N-β-Benzylmercaptoethyl-DL-isoleucine	B	95% ethanol	50[d]	232–233	$C_{15}H_{23}O_2NS$	64.0	8.24	64.0	8.63
N-β-Benzylmercaptoethyl-DL-serine	B	50% ethanol	15[d]	178–184	$C_{12}H_{17}O_3NS$	56.5	6.67	57.0	7.11
N-β-Benzylmercaptoethyl-DL-threonine	B	Water	30[d]	226	$C_{13}H_{19}O_3NS$	N 5.49		N 5.12	
N-β-Benzylmercaptoethyl-DL-phenylalanine	B	Water	40[d]	224–225	$C_{18}H_{21}O_2NS$	68.5	6.71	68.5	6.88
N-β-Benzylmercaptoethyl-DL-methionine	B	Acetic acid	20[d]	209–210	$C_{14}H_{21}O_2NS_2$	56.2	7.07	56.6	7.17
N,N-bis-(β-Benzylmercaptoethyl)-glycine	C	Benzene	4[b]	113–114	$C_{20}H_{25}O_2NS_2$	64.0	6.71	63.9	6.51
N,N-bis-(β-Benzylmercaptoethyl)-L-tryptophan	B	Ethanol–benzene	30[d]	185–188	$C_{29}H_{32}O_2N_2S_2$	69.0	6.39	69.1	6.46
N-β-Butylmercaptoethylglycine	C	Abs. ethanol	10[b]	175–180	$C_8H_{17}O_2NS$	N 7.32		N 7.36	
N-β-Butylmercaptoethyl-DL-leucine	B	50% ethanol	25[d]	260	$C_{12}H_{25}O_2NS$	N 5.66		N 5.59	
N-β-Butylmercaptoethyl-DL-phenylalanine	B	95% ethanol	35[d]	225–226	$C_{15}H_{23}O_2NS$	N 4.98		N 4.79	
N-β-Butylmercaptoethyl-L-tryptophan	B	Ethanol–benzene	5[d]	178–181	$C_{17}H_{23}O_2N_2S$	N 8.77		N 8.60	
N,N-bis-(β-butylmercaptoethyl)-glycine	C	Benzene	5[b]	87–98	$C_{14}H_{29}O_2NS_2$	N 4.55		N 4.40	

[a] The N-acetyl derivative was prepared in 75% yield and recrystallized from dilute alcohol; m. p. 148–149°. *Anal.* Calcd. for $C_{13}H_{17}O_3NS$: C, 58.4; H, 6.41. Found: C, 58.3; H, 6.48. [b] Based on amount of vesicant used. [c] The N-acetyl derivative was prepared in 80% yield and recrystallized from water; m. p. 120–121°. *Anal.* Calcd. for $C_{14}H_{19}O_3NS$: C, 59.8; H, 6.81. Found: C, 60.0; H, 6.43. [d] Based on amount of amino acid used.

A series of crystalline derivatives of the amino acids was obtained, demonstrating reaction of the one-handed vesicants with sulfhydryl, amino, phenolic hydroxyl and imidazolyl groups under the conditions employed. No evidence was found to indicate reaction with alcoholic hydroxyl, guanido or indolyl groupings. The methods of preparation and study of the compounds are presented not only for the intrinsic interest of the compounds themselves, but also for their possible importance in establishing the nature of protein vesicant reactions. In this latter capacity some of the derivatives can be considered as model substances and as reference compounds in cases where it is desirable to isolate the components of hydrolysates from vesicant-treated proteins.

Simple α-Amino Acids.—Other investigators[12] have demonstrated the facile esterification of the free carboxyl group of amino acid derivatives by H-type compounds. In our work we investigated the alkylation of amino groups with particular reference to the actual isolation of derivatives. It appeared likely that the amino groups would be susceptible to mono-, di- or trialkylation by the $RSCH_2CH_2$- radicals. In practice, treatment of the amino acids in alkaline solution did lead to the formation of a mixture of products from which it was possible to isolate crystalline Nα-alkyl derivatives, Nα-dialkyl derivatives in some instances, but no Nα-trialkyl derivatives (quaternary ammonium compounds). After N,N-bis-(β-benzylmercaptoethyl)-glycine was treated with benzyl-H, it was recovered unchanged in 80% of the theoretical yield. This indicates that the formation of a quaternary ammonium compound is probably not a major reaction under the experimental conditions. The alkylation of α-amino groups by H has been discussed in two recent papers[10,12c] covering work done in the war period by other laboratories.

The monosubstituted derivatives of glycine and alanine were readily acetylated in 75–80% yield by treatment in alkaline solution with acetic anhydride. In contrast, the monosubstituted derivatives of valine, leucine and phenylalanine were recovered unchanged in good yield. This difference in behavior held true both for the butyl-H and the benzyl-H series.

The several procedures for preparation of the derivatives are described in the Experimental part and pertinent data are compiled in Table I.

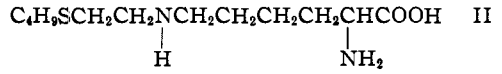
Lysine.—This amino acid was of particular interest since it is known[13] that the ε-amino group is reactive in many proteins. Kurtz[14] showed that treatment of the copper salt of

(12) (a) Bergmann, *et al.*, NRDC Section B4C Reports, April 25, 1942, and August 19, 1942; (b) Bergmann, *et al.*, OSRD Report, May 21, 1943; (c) Moore, Stein and Fruton, *J. Org. Chem.*, **11**, 675 (1946).

(13) Goldschmidt and Kinsky, *Z. physiol. Chem.*, **183**, 244 (1929); Gurin and Clark, *J. Biol. Chem.*, **107**, 395 (1934).

(14) Kurtz, *ibid.*, **140**, 705 (1941).

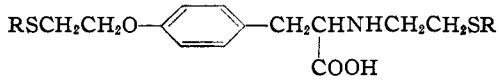
lysine in alkaline solution with benzoyl chloride yielded the N^{ϵ}-benzoyl derivative. The copper salt of lysine was, therefore, treated with butyl-H. A crystalline monosubstituted derivative of lysine was isolated. This is presumably the hydrochloride of structure II.



II

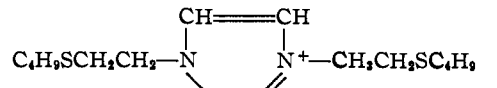
Mercaptoamino Acids.—We were interested particularly in preparing S-substituted vesicant derivatives of mercaptoamino acids because of the possible reactivity of protein sulfhydryl groups with vesicants.[15] The benzyl-H derivatives of the sulfhydryl group of cysteine and homocysteine were obtained in good yield by alkylation with the aid of sodium and liquid ammonia.[16]

Tyrosine.—The phenolic hydroxyl group of tyrosine is stated to be chemically reactive in proteins.[17] The reaction of this group with the vesicants was, therefore, of interest. Treatment of L-tyrosine in strongly alkaline solution with either benzyl-H or butyl-H yielded a mixture of products from which was isolated in each case a disubstituted derivative in 20% yield. The compounds show negative tests for phenolic hydroxyl groups[18] and are, therefore, believed to be O,N-disubstituted derivatives.

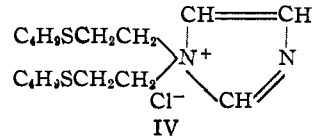


Histidine.—Preliminary studies of the reaction of vesicants with L-histidine and with N^{α}-benzoyl-L-histidine were made using a colorimetric method[19] based on the Pauly diazo reaction.[20] The results indicated a reaction of the vesicant with the imidazole ring. Evidence had been obtained by Moritz and co-workers[21] that the imidazole group of histidine reacted with H, and Ball and co-workers[22] suggested that the effect of H on the oxygen dissociation curve of hemoglobin might be interpreted as indicating a reaction with the imidazole groups of histidine in the intact protein.

We, therefore, studied the reaction of histidine and its derivatives further. Treatment of L-histidine in 0.5 *M* sodium bicarbonate solution with an excess of butyl-H yielded a crystalline substance, the composition of which corresponded to a trialkyl derivative. Treatment of imidazole under similar conditions yielded a disubstituted derivative. By analogy with other alkylation products of imidazole[23] structure III was considered likely for the imidazole derivative, although dialkylation of one N (IV) was not excluded. The

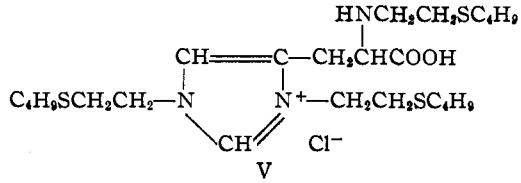


III



IV

structure analogous to structure III, in the case of the histidine derivative, would be structure V.



V

The compound yields no nitrogen in the Van Slyke procedure for the estimation of free amino nitrogen,[24] and is stable to heating under reflux in strong acid.

Using a smaller quantity of butyl-H under slightly different conditions, it was also possible to isolate a monosubstituted derivative of imidazole, and monoalkylation of the imidazole nitrogen alone was also apparently achieved by using N^{α}-benzoylhistidine.

Experimental[25,26]

Derivatives of Simple α-Amino Acids. These derivatives were prepared by one of the following procedures: **A.**—The amino acid (1 mole) was dissolved in 50% ethanol containing 3 moles of sodium carbonate or sodium hydroxide and treated with 2 moles of vesicant. The mixture was stirred for several hours at 30–50°, and then extracted with ether. Neutralization of the aqueous solution with hydrochloric acid precipitated the crude derivative which was recrystallized from the appropriate solvent.

B.—The amino acid was dissolved in slightly more than 3 equivalents of 1 *N* sodium hydroxide in 95% methanol. Two equivalents of vesicant were added and the mixture was allowed to stand for at least twenty-four hours. The solution was then decanted from the precipitated sodium chloride and concentrated *in vacuo*. The residue was dissolved in water. The solution was extracted with ether and then neutralized with hydrochloric acid. The precipitated derivative was collected and recrystallized from the appropriate solvent.

(15) Investigations of vesicant-protein sulfhydryl reactions have been discussed elsewhere: (a) Hellerman, final summarization of NDRC work, Contract OEMsr-94; (b) Banks, Boursnell, Francis, Hopwood and Wormall, *Biochem. J.*, **40**, 745 (1946).

(16) du Vigneaud, Audrieth and Loring, THIS JOURNAL, **52**, 4500 (1930).

(17) See, for instance, Herriott, *J. Gen. Physiol.*, **19**, 283 (1938); Rutherford, Patterson and Harris, *J. Research Natl. Bur. Standards*, **25**, 451 (1940).

(18) Folin and Ciocalteu, *J. Biol. Chem.*, **73**, 627 (1927).

(19) Macpherson, *Biochem. J.*, **36**, 59 (1942).

(20) Pauly, *Z. physiol. Chem.*, **44**, 159 (1905).

(21) Moritz, Henriques, *et al.*, Progress Report to Division 9, NDRC, August 28, 1942.

(22) Ball, Davis and Ross, Progress Report to Division 9, NDRC, December 19, 1942; Davis and Ross, THIS JOURNAL, **69**, 1177 (1947).

(23) Pinner and Schwarz, *Ber.*, **35**, 2441 (1902).

(24) Van Slyke, *J. Biol. Chem.*, **12**, 275 (1912).

(25) All melting points were determined on a calibrated hot stage.

(26) The authors are indebted to Dr. Julian R. Rachele and Mr. Roscoe C. Funk, Jr., for the microanalyses.

C.—The amino acid was dissolved in 1 equivalent of 1 *N* sodium hydroxide in 95% methanol and an equal volume of water was added. The vesicant (0.25 equivalent) was added, the mixture was stirred until homogeneous and then was allowed to stand overnight. When glycine was treated under these conditions and the reaction products were isolated by the method described under procedure B, crystalline disubstituted derivatives were obtained. Concentration of the mother liquors to a small volume yielded crystalline monosubstituted derivatives.

The methods of preparation and the properties of the various derivatives are recorded in Table I.

It seems certain that the derivatives prepared from L-tryptophan involve the α-amino group.[27] To check the possibility of a reaction of the indole group with the vesicants, the α-amino group was covered by acetylation. No evidence was found of the reaction of the vesicants with the indole group of N^{α}-acetyl-L-tryptophan.

Derivative of Lysine.—L-Lysine dihydrochloride (2.17 g.) was dissolved in 50 cc. of 2.5% sodium tetraborate. Copper carbonate was added in excess and the solution was heated to boiling and filtered. The solution was brought to *p*H 9.2 with 5 *N* sodium hydroxide and 4 g. of sodium tetraborate was added. Butyl-H (1.7 cc.) was added, and the solution was stirred for twenty-four hours. Then the precipitated copper salt was filtered and washed with water. A suspension of the copper salt (*p*H 9) was treated with hydrogen sulfide and, after removal of copper sulfide, the solution was acidified to litmus with acetic acid. The solvent was removed *in vacuo* leaving a sirup which crystallized on addition of concentrated hydrochloric acid. The material was recrystallized three times from a minimum amount of hot water by addition of ethanol. The yield of this purified product decomposing at 240° was 0.46 g. (15% of the theoretical amount).

Anal. Calcd. for $C_{12}H_{26}O_2SN_2 \cdot HCl$: S, 10.73; Cl, 11.86. Found: S, 10,33; Cl, 11.25.

Derivatives of Cysteine and Homocysteine.—L-Cystine (24 g.) and sodium were added in portions to 400 cc. of liquid ammonia, just enough sodium being added at the end to give a blue color persisting for ten minutes. Then 32.4 cc. of benzyl-H was added dropwise. The liquid ammonia was allowed to evaporate. The solid residue was stirred well with crushed ice until the ice melted. The resulting precipitate was collected. The filtrate was extracted with ether and then neutralized with concentrated hydrochloric acid. A second precipitate resulted. The combined precipitates were recrystallized from hot water. The yield was 25 g. of material melting at 187–189° and having the composition of the expected S-(β-benzylmercapto)-ethylcysteine.

Anal. Calcd. for $C_{12}H_{17}O_2NS_2$: C, 53.1; H, 6.31. Found: C, 53.3; H, 6.21.

The compound was characterized further by conversion to the acetyl derivative which melted at 125° and had the expected neutral equivalent (313).

Under similar conditions, DL-homocystine yielded S-(β-benzylmercapto)-ethylhomocysteine, m. p. 225°.

Anal. Calcd. for $C_{13}H_{19}O_2NS_2$: N, 4.91; S, 22.46. Found: N, 4.60; S, 22.79.

This compound was characterized further by conversion to the acetyl derivative which melted at 70–72°. The neutral equivalent of the acetyl derivative was 324 (calculated, 327).

Derivatives of Tyrosine.—Experiments indicated that the vesicants did not react appreciably with phenolic groups in neutral solution. However, in a strongly alkaline solution it was possible to prepare O,N-disubstituted derivatives of tyrosine. L-Tyrosine (1.81 g.) was shaken for twenty-four hours with 40 cc. of 1 *N* sodium hydroxide, 40 cc. of methanol, and 4.5 cc. of butyl-H. The reaction product was then isolated according to procedure B. An amorphous product weighing 2.4 g. was obtained. On recrystallization from 70% acetic acid, 1.0 g. of a crystalline derivative melting at 208–210° was obtained. The compound gave a negative Millon test, and yielded no color with Folin phenol reagent.[18]

Anal. Calcd. for $C_{21}H_{35}O_3NS_2$: N, 3.39. Found: N, 3.34.

Treatment of L-tyrosine (1.81 g.) with benzyl-H (procedure B) yielded an amorphous product weighing 3.1 g. This product was dissolved in 150 cc. of 50% ethanol containing 3 g. of sodium bicarbonate. The cooled solution deposited 1.0 g. (20%) of the pure O,N-disubstituted derivative. It gave a negative Millon's test and melted at 203–205°. It formed plates on recrystallization from 80% acetic acid.

Anal. Calcd. for $C_{27}H_{31}O_4NS_2$: C, 67.3; H, 6.48. Found: C, 67.6; H, 6.54.

Derivatives of Histidine and Related Compounds. L-Histidine.—L-Histidine monohydrochloride monohydrate (10.2 g.) was dissolved in 500 cc. of 0.5 *M* sodium bicarbonate and the solution was stirred vigorously with 36 cc. of butyl-H for twenty-four hours. About 30 cc. of a brown oil separated. This material was extracted three times with 150-cc. portions of ether. The ether-insoluble residue was diluted with 3 volumes of chloroform, and the solution was washed with 10% hydrochloric acid and with water. Evaporation of the chloroform left a sirupy residue which did not crystallize. The sirup was dissolved in chloroform (80 cc.) and stirred for half an hour with Brockmann alumina. The mixture was filtered and the chloroform was removed *in vacuo*. Addition of 10 cc. of acetone to the residue caused slow crystallization.[28] The yield of crystalline material was 0.9–1.4 g. Several recrystallizations from methylene chloride gave a sample melting at 187–188° and having the composition of a trisubstituted histidine derivative.

Anal. Calcd. for $C_{24}H_{46}O_2N_3S_3Cl$: C, 53.4; H, 8.58; N, 7.78; S, 17.80; Cl, 6.56. Found: C, 53.4; H, 7.87; N, 7.65; S, 18.05; Cl, 6.41.

The derivative was recovered quantitatively after being heated in water at 100° for twenty-four hours, and in 75% yield after treatment for twelve hours in refluxing 20% hydrochloric acid.

Imidazole.—Imidazole (0.136 g.) and butyl-H (0.153 g.) were dissolved in 218 cc. of 0.7 *N* potassium hydroxide in 95% methanol. After the solution had been allowed to stand overnight, another 0.153 g. of butyl-H was added, and the resulting mixture was allowed to stand for four hours. The solvents were removed *in vacuo*, 2 cc. of water was added to the residue, and the solution was extracted with 5 cc. of benzene. The benzene layer was evaporated *in vacuo* and 6 cc. of water was added to the residue. A saturated aqueous solution of picrolonic acid was added to the aqueous solution. Seventy-two milligrams of yellow crystalline material melting at 140–155° (dec.) was obtained. The compound, after recrystallization from water, melted at 154–156° (dec.) and had the composition of the picrolonate of a monosubstituted imidazole derivative.

Anal. Calcd. for $C_9H_{16}N_2S \cdot C_{10}H_8O_5N_4$: C, 50.9; H, 5.39. Found: C, 50.0; H, 5.66.

A disubstituted derivative of imidazole was prepared as follows: A solution of imidazole (1.36 g.) in 300 cc. of 0.5 *M* sodium bicarbonate was stirred with 15 cc. of butyl-H for twenty-four hours. The mixture was acidified to congo red with concentrated hydrochloric acid and extracted 3 times with 50-cc. portions of ether. The

(27) In the course of the study of these derivatives, it was observed that the disubstituted derivative of L-tryptophan gives approximately twice the color given by an equimolar amount of L-tryptophan when treated with the Folin phenol reagent.[18] Further study showed that all of the N^{α}-disubstituted vesicant derivatives of the amino acids gave a blue color with the reagent.

(28) If the material did not crystallize at this point, it was washed with ether, dissolved in 30 cc. of methylene chloride and washed with water. Evaporation of the solvent left a sirup which crystallized on addition of acetone.

aqueous solution was then extracted 3 times with chloroform. The combined chloroform extracts were dried over anhydrous magnesium sulfate. The chloroform solution upon evaporation gave a light yellow oil which crystallized upon addition of ether. The yield was 0.23 g., and additional material was obtained by further chloroform extractions. For analysis the crude product was crystallized from a mixture of benzene and acetone. After the product had been dried at 3 mm. over potassium hydroxide for twenty minutes, it had the approximate composition of a monohydrate, m. p. 50–52°.

Anal. Calcd. for $C_{15}H_{29}N_2S_2Cl \cdot H_2O$: C, 50.8; H, 8.80. Found: C, 51.4; H, 8.62.

On long drying *in vacuo* at 40°, the above material lost 93% of the calculated weight for 1 molecule of water of hydration. It then melted at 56–57°.

Anal. Calcd. for $C_{15}H_{29}N_2S_2Cl$: C, 53.5; H, 8.67; N, 8.31; S, 19.03; Cl, 10.52. Found: C, 53.1; H, 8.79; N, 8.00; S, 19.50; Cl, 10.14.

N^{α}-Benzoyl-L-histidine.—Three grams of N^{α}-benzoyl-L-histidine[29] was dissolved in 25 cc. of water by addition of 1 *N* sodium hydroxide with stirring. Then 1.5 cc. of butyl-H was added, and the mixture was stirred for five hours. The *p*H was maintained at 8–9 by gradual addition of 1 *N* sodium hydroxide. Methanol (10 cc.) was also added portionwise to increase the solubility of the vesicant. The reaction mixture was evaporated to about one-half volume *in vacuo* and then extracted with ether. The aqueous layer was acidified to *p*H 4 with 11 cc. of 1 *N* hydrochloric acid. The oil which separated was removed. The aqueous solution was acidified with 1 cc. of 1 *N* hydrochloric acid and extracted with chloroform. Evaporation of the chloroform left an oil which was crystallized from ethanol by addition of ether. Recrystallization of the compound from water yielded 200 mg. of rosettes, m. p. 188–190°.

Anal. Calcd. for $C_{19}H_{25}O_3N_3S$: S, 8.54. Found: S, 8.37.

Summary

1. A series of N-substituted derivatives of the simple α-amino acids with benzyl-H (benzyl β-chloroethyl sulfide) and butyl-H (butyl β-chloroethyl sulfide) has been prepared by treatment of the various amino acids in alkaline solution with the corresponding vesicant. The following derivatives have been prepared: N-monosubstituted benzyl-H derivatives of glycine, DL-alanine, DL-valine, L-leucine, DL-leucine, DL-isoleucine, DL-threonine, DL-phenylalanine and DL-methionine; N^{α}-monosubstituted butyl-H derivatives of glycine, DL-leucine, DL-phenylalanine and L-tryptophan; N^{α}-disubstituted benzyl-H derivatives of glycine and L-tryptophan; N^{α}-disubstituted butyl-H derivative of glycine.

2. Treatment of the copper salt of L-lysine in alkaline solution with butyl-H yielded a crystalline monosubstituted derivative. By analogy with benzoylation data this compound is believed to be the N^{ϵ}-substituted derivative.

3. S-Substituted derivatives of L-cysteine and DL-homocysteine with benzyl-H have been prepared by reaction of the vesicant with the corresponding sodium mercaptides in liquid ammonia solution.

4. O,N-Disubstituted derivatives of L-tyrosine with benzyl-H and butyl-H have been prepared.

5. A trisubstituted butyl-H derivative of L-histidine was obtained by treatment of the amino acid in alkaline solution with the vesicant. Under similar conditions imidazole yielded mono- and disubstituted derivatives, and N^{α}-benzoyl-L-histidine yielded a monosubstituted derivative.

6. The data provide a further and direct demonstration that the following groups in amino acids are capable of reacting with H-type vesicants: α-amino group, ε-amino group, imidazolyl group, sulfhydryl group and phenolic hydroxyl group.

(29) Gerngross, *Z. physiol. Chem.*, **108**, 50 (1919).

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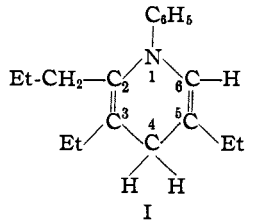
N-Phenyl-3,5-diethyl-2-propyl-1,4-dihydropyridine

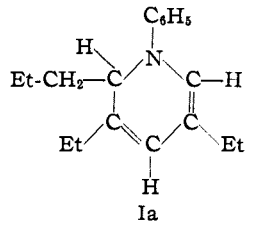
BY DAVID CRAIG, LAURA SCHAEFGEN AND WILLARD P. TYLER

During a study of the reaction of butyraldehyde with aniline in the presence of acetic acid, it has been found possible to isolate a weak base having the formula $C_{18}H_{25}N$. The present paper deals with the structure of this base.

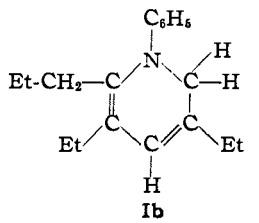
The empirical formula and the method of synthesis suggest dihydropyridine structures I, Ia, or Ib or the open chain anil structure II. The pyrolysis of the compound in the presence of cobaltous chloride forms aniline and 1,3,5-triethylbenzene. The formation of triethylbenzene, $C_{12}H_{18}$, supports these formulations since the linking together of three butyraldehyde residues is thereby indicated. Hydrogenation, depending on conditions, yields di, tetra and decahydro derivatives, in accord with the N-phenyldihydropyridine formulas, but thus far has given no evidence of the



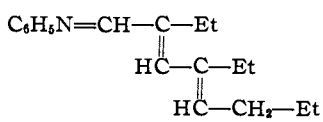
I



Ia



Ib



II