Isolation of Harmine.—Direct crystallization of (B) from hot chloroform (with concentration) yielded 5 g. of crude harmine. A similar crystallization of (A) yielded 3 g. of crude harmine. These two lots were combined and recrystallized twice from methanol to yield 5 g. (0.3%) of pure harmine, m.p. 261.8–262.4°; mixture m.p. with an authentic sample[8] not depressed. The infrared absorption spectrum and the paper chromatographic behavior were identical with that of the pure synthetic sample.

Isolation of Harmaline and *d*-Tetrahydroharmine.—A second crop of alkaloids (2 g.) obtained by concentration of the mother liquors from A and B above, contained, in addition to harmine, larger quantities of two other alkaloids, which were detected readily by their paper chromatographic behavior. On a chloroform–formamide system,[9] harmine shows R_f 0.64, harmaline has R_f = 0.07, and tetrahydroharmine has R_f 0.13. All three compounds fluoresce brightly under ultraviolet light. Attempts to separate the three components by an 8-tube countercurrent distribution between chloroform and *p*H 4.6 phosphate buffer showed limited separation. The crude mixture sublimed completely at 185° (0.1 mm.). Chromatography of 0.5 g. of this mixture over 30 g. of "Florisil," utilizing chloroform with increasing increments of methanol, finally pure methanol for elution, yielded first harmine, then tetrahydroharmine, finally harmaline.

The tetrahydroharmine, of which about 50 mg. (0.003%) was isolated in pure form, had m.p. 198.4–199.8° (*in vacuo*), $[\alpha]^{25}D$ +32° ($CHCl_3$, or 5% acetic acid). The mixed m.p. with racemic tetrahydroharmine,[10] m.p. 199.4–199.8° (*in vacuo*) was not visibly depressed. Further, the infrared spectra of the natural and synthetic materials were identical, both in chloroform solution and in KBr pellets. The papergram characteristics of the two forms were indistinguishable.

Harmaline (25 mg.), the last component eluted from the column, was purified by sublimation, and melted at 227–229°. The mixture m.p. with an authentic sample, prepared from a commercial product, m.p. 228.5–231°, was not depressed. Identity was confirmed by ultraviolet and infrared absorption spectra, and by paper chromatographic comparison.

An aqueous extract of *B. caapi*, "as used by the natives," was supplied to us at a different time. It contained the same three alkaloids, but it appeared to be richer in *d*-tetrahydroharmine and harmaline. One liter of this extract contained 3.3 g. of chloroform extractable solids, from which about one gram of pure harmine was isolated.

Isolation of Dimethyltryptamine from *Prestonia amazonicum* Extract.—The aqueous extract of the leaves was received as a turbid suspension, *p*H 5.5, with a faintly aromatic odor. Two liters of extract were adjusted to *p*H 10.5 with sodium hydroxide, and extracted with three one-liter portions of chloroform. The chloroform extracts were washed with water, and concentrated *in vacuo* to yield 4.3 g. of a viscous oil which was readily soluble in dilute acetic acid or mineral acid. Distillation at 170° (0.01 mm.) yielded 3 g. of colorless oil which crystallized spontaneously on standing, m.p. 44–46°. A second distillation yielded purified N,N-dimethyltryptamine, m.p. 44.6–46.8°, pK_a (ethanol–water), 8.68, eq. wt. 190, calcd. 188. The identity, which was immediately suspected from the analysis and the ultraviolet absorption spectrum, was established by preparation of the picrate, m.p. 169.5–170°, and of the methiodide, m.p. 216–217°.

Examination of the crude total alkaloids by paper chromatography revealed only dimethyltryptamine.

N,N-Dimethyl-6-methoxytryptamine.—Oxalyl chloride (0.5 g., 6 mmoles) was added to a solution of 0.44 g. (3 mmoles) of 6-methoxyindole[11] in 15 ml. of ether at 0°. An orange-red crystalline precipitate separated within a few minutes. After 30 minutes the crude 6-methoxy-3-indoleglyoxychloride, 0.66 g., was separated by filtration, washed with ether and added with vigorous stirring to 20 ml. of 35% aqueous dimethylamine. After 15 minutes the clear yellow solution was concentrated *in vacuo* to a few ml., and 0.57 g. of crystalline 6-methoxyindoleglyoxyldimethylamide separated and dried, m.p. 194–195°. A portion, 75 mg., was recrystallized from 15 ml. of benzene to yield 55 mg. of pure compound, m.p. 195.1–196.8°.

Anal. Calcd. for $C_{13}H_{14}N_2O_3$: C, 63.40; H, 5.73; N, 11.38. Found: C, 63.00; H, 5.79; N, 11.53.

One-half gram of the crude amide was dissolved in 35 ml. of dry tetrahydrofuran, 0.50 g. of lithium aluminum hydride in 20 ml. of tetrahydrofuran added, and the resulting suspension was heated under reflux for three hours. The excess hydride was decomposed with 100 ml. of water and the product extracted several times with methylene chloride. Concentration of the combined, water-washed extracts yielded 0.47 g. of colorless crystals, of crude N,N-dimethyl-6-methoxytryptamine, m.p. 68–75°. Two recrystallizations from ligroin yielded a pure product, m.p. 76–77.2°.

Anal. Calcd. for $C_{13}H_{18}N_2O$: C, 71.52; H, 8.31; N, 12.83. Found: C, 71.18; H, 8.00; N, 13.40.

The ultraviolet absorption spectra, which show peaks in methanol at 223 mμ, ε 21,200, 273 mμ, ε 2600 and 294 mμ, ε = 3180, is very similar but not identical with those of such α,β-disubstituted 6-methoxyindoles as tetrahydroharmine or methyl reserpate.

Acknowledgments.—We should like to express our thanks to Mr. W. H. Boegemann for his invaluable assistance with paper chromatography. We are indebted also to Dr. R. L. Wagner and his colleagues for the physical measurements and analytical data, and to Dr. L. Nickell for his continuing help with the botanical aspects of the problem.

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(8) The authentic sample was prepared by the nitric acid oxidation of commercial (E. Merck, Darmstadt) harmaline by the procedure of V. V. S. Iyer and R. Robinson, *J. Chem. Soc.*, 1635 (1934).

(9) F. A. Hochstein, K. Murai and W. H. Boegemann, THIS JOURNAL, **77**, 3551 (1955).

(10) Tetrahydroharmine was prepared by the sodium–amalgam reduction of harmaline hydrochloride, following the procedure of W. H. Perkin and R. Robinson, *Chem. Soc.*, **115**, 961 (1919), for the reduction of harmine.

(11) We are indebted to Dr. R. B. Woodward for this material.

[CONTRIBUTION FROM THE DEPARTMENT OF ORGANIC CHEMISTRY, WEIZMANN INSTITUTE OF SCIENCE]

Reaction of Acylamino Acids with Paraformaldehyde

BY DOV BEN-ISHAI*

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Carbobenzoxy derivatives of simple α-amino acids, hippuric and phenaceturic acids have been found to react with paraformaldehyde in the presence of a sulfonic acid catalyst to give N-acyl-5-oxazolidones (II). The reaction of the oxazolidinones with amines is described.

Primary amides and primary urethans are known to react with formaldehyde or paraformaldehyde under acidic conditions to give methylenediamides (I) and methylenediurethans[1] (I, R = OR_1)

$$2RCONH_2 + CH_2O \xrightarrow{H^+} (RCONH)_2CH_2 + H_2O$$
$$\text{I}$$

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(1) J. F. Walker, "Formaldehyde," Amer. Chem. Soc., Monograph Series, Reinhold Publ. Corp., New York, N. Y., 1944, Chapter 14.

TABLE I

N-ACYL-5-OXAZOLIDINONE (II)

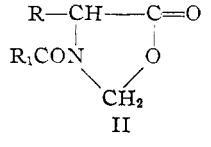
R	R_1	M.p., °C.	Yield, %	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
H	C_6H_5	151	74	62.82	62.94	4.75	4.83	7.33	7.22
H	$C_6H_5CH_2$	113	81	64.38	64.67	5.40	5.38	6.83	7.01
H	$C_6H_5CH_2O$	84	64	59.73	59.90	4.97	5.00	6.33	6.56
CH_3	$C_6H_5CH_2O$	65	72	61.27	61.40	5.57	5.51	5.96	6.13
$(CH_3)_2CH$[a]	$C_6H_5CH_2O$	54	41	63.86	64.10	6.51	6.36	5.32	5.33
$(CH_3)_2CHCH_2$[b]	$C_6H_5CH_2O$	63	62	64.96	64.91	6.91	6.85	5.05	5.25
$C_6H_5CH_2$[c]	$C_6H_5CH_2O$	83	80	69.44	69.66	5.50	5.51	4.50	4.55

[a] $[\alpha]^{20}D$ +95° (*c* 1 in chloroform). [b] $[\alpha]^{20}D$ +108° (*c* 2 in chloroform). [c] $[\alpha]^{20}D$ +212° (*c* 1 in chloroform).

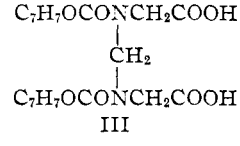
When functional groups other than the amide are present in the molecule, intramolecular methylene bridge formation may occur leading to cyclic products. Thus, asparagine affords, on treatment with formaldehyde, 6-hydroxy-tetrahydro-pyrimidyl-4-carboxylic acid.[2]

In the present paper the reaction of paraformaldehyde with acylamino acids, which may be considered as substituted amides or substituted urethans having an additional carboxy function, is described. Heating a mixture of carbobenzoxyamino acids with paraformaldehyde in benzene solution under reflux in presence of a sulfonic acid catalyst for 0.5 hr. affords crystalline neutral compounds (Table I) as well as acidic products (*e.g.*, III).

The analytical data and the infrared spectra of the neutral components are consistent with the 5-oxazolidinone formulation (II, $R_1 = C_6H_5CH_2O$). The neutral compounds lack the carboxy OH absorption and the bands at 3440 and 1510 cm.$^{-1}$, characteristic of carbamate NH, present in the starting materials and show instead new sharp and strong carbonyl absorption in the 1800–1815 cm.$^{-1}$ region characteristic of 5-oxazolones.[3] N-Carbobenzoxy-2-oxazolidinone which is isomeric with II (R = H; $R_1 = C_6H_5CH_2O$) shows similar carbonyl absorptions at 1720 and 1800 cm.$^{-1}$ in the infrared spectrum.[4]



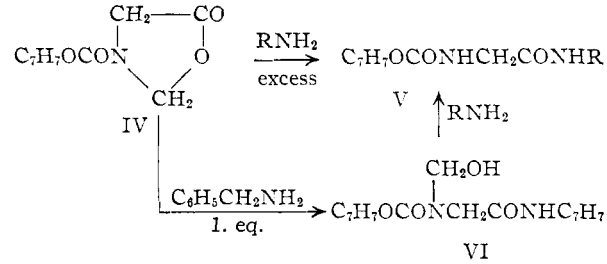
II



III

N-Carbobenzoxy-5-oxazolidinone (IV) prepared from carbobenzoxyglycine reacts with excess ammonia or with benzylamine in alcoholic solution to give carbobenzoxyglycineamide (V, R = H) and carbobenzoxyglycine benzylamide (V, R = $C_6H_5CH_2$), respectively. This conversion takes place at room temperature within 5 hr. and affords the corresponding amides in 96 and 87% yield, respectively.

If carbobenzoxy-5-oxazolidinone (IV) is treated with only one equivalent of benzylamine, a crystalline compound is obtained, which on further treatment with ammonia or benzylamine is converted to carbobenzoxyglycine benzylamide (V, R = $C_6H_5CH_2$). The analytical data, the infrared spectrum and the fact that this intermediate yields sarcosine benzylamide on catalytic hydrogenation strongly supports the N-methylol-N-carbobenzoxyglycine benzylamide formulation (VI).



VI

The acidic material obtained in the reaction of carbobenzoxyglycine and paraformaldehyde and for which formula III is suggested is a normal product in the reaction of carbamates with formaldehyde.[1] The same acidic product was also obtained by treating ethyl carbobenzoxyglycinate with paraformaldehyde and subsequent hydrolysis of the first formed oily diester.

Oxazolidones (II) were also prepared from carbobenzoxy derivatives of *dl* alanine, *l*-valine, *l*-leucine and *l*-phenylalanine (Table I) (the acidic products were not characterized in these cases). They all show the characteristic carbonyl bands at 1710 and 1800 cm.$^{-1}$. The oxazolidinones from optically active carbobenzoxyamino acids possess high optical rotations. Optically active N-carbobenzoxy-4-benzyl-5-oxazolidinone (II, R = $C_6H_5CH_2$; R_1 = $C_6H_5CH_2O$) could be converted, on treatment with excess ammonia, to optically active carbobenzoxyphenylalanineamide, identical with an authentic sample prepared from carbobenzoxy-*l*-phenylalanyl chloride and ammonia.

Since hippuric and phenaceturic acids are insoluble in benzene, they were converted to the corresponding oxazolidinones (Table I) on treating with paraformaldehyde in 1,1,2-trichloroethane solution. They both show the characteristic oxazolidinone carbonyl absorptions at 1810 cm.$^{-1}$ and the amide carbonyl absorptions at 1645 and 1665 cm.$^{-1}$, respectively.

Experimental[5]

Reaction of Carbobenzoxyamino Acids with Paraformaldehyde.—A mixture of carbobenzoxyamino acid (0.025 mole), paraformaldehyde (1 g.) and *p*-toluenesulfonic acid (250 mg.) in benzene (200 ml.) was refluxed for 0.5 hr., the water being distilled off and collected in a water separator

(2) D. French and J. T. Edsall, *Advances in Protein Chem.*, **2**, 306 (1945).

(3) H. T. Clark, J. R. Johnson and R. Robinson, editors, "The Chemistry of Penicillin," Princeton University Press, Princeton, N. J., 1949, p. 400.

(4) D. Ben-Ishai, THIS JOURNAL, **78**, 4962 (1956).

(5) All melting points are uncorrected.

as it was formed. The benzene solution was washed with 5% aqueous potassium bicarbonate solution and then dried over sodium sulfate. The N-carbobenzoxy-5-oxazolidones obtained, after removal of the benzene *in vacuo*, were crystallized from petroleum ether or benzene light petroleum (Table I).

Methylenebiscarbobenzoxyglycine (III).—The bicarbonate solution obtained above in the reaction of carbobenzoxyglycine with paraformaldehyde was acidified with 5% hydrochloric acid and extracted with ether. The ether solution was washed with water, dried over sodium sulfate and evaporated to dryness. The oily residue slowly crystallized on trituration with a small amount of benzene. The product melted at 114–115°, and this m.p. was depressed on admixture with carbobenzoxyglycine (102–108°); yield 24%.

Anal. Calcd. for $C_{21}H_{22}N_2O_8$: C, 58.60; H, 5.15; N, 6.51. Found: C, 58.69; H, 5.30; N, 6.40.

Methylenebiscarbobenzoxyglycine from Carbobenzoxyglycine Ethyl Ester.—A solution of ethyl carbobenzoxyglycinate (12 g.) in benzene (200 ml.) was heated under reflux with paraformaldehyde (2 g.) and *p*-toluenesulfonic acid (300 mg.), the water being removed azeotropically. After the theoretical amount of water had distilled off (1 hr.), the benzene solution was washed with 5% aqueous bicarbonate solution, dried over sodium sulfate and evaporated *in vacuo* to dryness.

The oily diester (12.5 g.) was dissolved in 150 ml. of 2% alcoholic potassium hydroxide and the solution allowed to stand at room temperature for 1 hr. After removal of the ethanol the residue was dissolved in water (100 ml.), extracted with ether and the aqueous solution acidified with hydrochloric acid 5%. The oil which separated was extracted with ether, dried over sodium sulfate and the ether evaporated. The residue slowly crystallized after trituration with benzene; yield 7 g. (65%). The methylenebiscarbobenzoxyglycine thus obtained melted at 114–115° and this m.p. was not depressed on admixture with compound III. The infrared spectra of the two were identical and lack the NH absorptions at 3440 and 1510 cm.$^{-1}$ present in the starting material (carbobenzoxyglycine).

Reaction of Hippuric Acid with Paraformaldehyde.—A mixture of hippuric acid (4.5 g.), paraformaldehyde (1 g.) and *p*-toluenesulfonic acid (250 mg.) in alcohol free 1,1,2-trichloroethane (200 ml.) was heated under reflux for 2 hr. and the water formed collected in a water separator. The solution was evaporated under reduced pressure and the residue dissolved in ether. The ethereal solution was washed with 5% bicarbonate solution, water and dried over sodium sulfate. The N-benzoyl-5-oxazolidinone obtained after removal of the ether was crystallized from benzene–petroleum ether (Table I).

The same procedure was used to prepare N-phenylacetyl-5-oxazolidinone.

Reaction of N-Carbobenzoxy-5-oxazolidinone (IV) with Ammonia.—The oxazolidinone (2.21 g.) was dissolved in ethanol (25 ml.), concentrated aqueous ammonia (3 ml.) was added and the solution was left for 5 hr. at room temperature. The ethanol was removed *in vacuo* and the residue crystallized from aqueous ethanol; yield 2 g. (96%). The carbobenzoxyglycineamide thus obtained melted at 136–137° and this m.p. was not depressed on admixture with an authentic sample prepared from ethyl carbobenzoxyglycinate and ammonia.[6]

Reaction of N-Carbobenzoxy-4-benzyl-5-oxazolidinone with Ammonia.—The procedure described above was repeated using 3.11 g. of the oxazolidone suspended in ethanol (25 ml.) and concentrated aqueous ammonia (3 ml.). The carbobenzoxyphenylalanine amide obtained melted at 167° after crystallization from ethyl acetate–petroleum ether, and this m.p. was not depressed on admixture with a sample prepared from carbobenzoxy-*l*-phenylalanyl chloride and ammonia; yield 2.5 g. (83%), $[\alpha]^{20}D$ +12.0° (*c* 1 in chloroform). The authentic sample showed the same specific rotation.

Reaction of N-Carbobenzoxy-5-oxazolidinone with Benzylamine (Excess).—The procedure described above using 2.21 g. of the oxazolidinone, 25 ml. of ethanol and 5 ml. of benzylamine was repeated. Carbobenzoxyglycine benzylamide thus obtained melted at 118–119° after crystallization from ethyl acetate–petroleum ether, and this m.p. was not depressed on admixture with a sample prepared from ethyl carbobenzoxyglycinate and benzylamine; yield 2.6 g. (87%).

N-Methylol-N-carbobenzoxyglycine Benzylamide (VI).—A mixture of N-carbobenzoxy-5-oxazolidinone (2.21 g.) and benzylamine (1.07 g.) in ethanol (25 ml.) was left overnight. The ethanol was removed *in vacuo* and the residue crystallized from benzene–petroleum ether. The product melted at 117–118°; yield 3 g. (91%). A mixed m.p. with carbobenzoxyglycine benzylamide (V, R = C_7H_7) was depressed (95–104°) and the infrared spectra were different. The methylol derivative showed OH absorptions at 3340 and 1025 cm.$^{-1}$ and carbonyl absorptions at 1695 and 1675 cm.$^{-1}$. Carbobenzoxyglycine benzylamide showed NH absorption at 3440 and 1510 cm.$^{-1}$ (strong) and two carbonyl absorptions at 1717 and 1675 cm.$^{-1}$.

Anal. Calcd. for $C_{18}H_{20}N_2O_4$: C, 65.84; H, 6.14; N, 8.53. Found: C, 66.03; H, 5.98; N, 8.81.

Sarcosine. Benzylamide Hydrochloride.—N-Methylol-N-carbobenzoxyglycine benzylamide (3 g.) was dissolved in ethanol (50 ml.), concentrated hydrochloric acid (1 ml.) was added and the solution was catalytically hydrogenated under 4 atm. pressure and in presence of 10% palladized charcoal (0.25 g.). After 3 hr. the solution was filtered from the catalyst and evaporated *in vacuo* to dryness. The residue which crystallized on trituration with dry ether was filtered and washed with ether; yield 1.8 g. (92%). The product, after drying *in vacuo* over potassium hydroxide, melted at 139–141°.

Anal. Calcd. for $C_{10}H_{15}N_2OCl$: N, 13.04; Cl, 16.53. Found: N, 13.21; Cl, 16.62.

N-Carbobenzoxysarcosine Benzylamide.—The sarcosine benzylamide obtained above was carbobenzoxylated by the Schotten–Baumann procedure. The product melted at 116–117° after crystallization from ethyl acetate–petroleum ether, and this m.p. was not depressed on admixture with a sample prepared from carbobenzoxysarcosyl chloride and benzylamine. The infrared spectra of the two were identical.

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(6) S. W. Fox and H. Fox, THIS JOURNAL, **73**, 2936 (1951).