3000 and 2960 (aliphatic CH); 1620 (NH); 1600, 1560 and 1545 (C=C, C=N); 1400 (unassigned).

Anal. Calcd. for $C_6H_9N_5$: C, 43.15; H, 6.52; N, 50.33. Found: C, 42.97; H, 7.01; N, 50.08.

Hydrogenolysis of 5-Amino-4-(1-benzylhydrazino)-6-chloropyrimidine. A. Raney Nickel.—Raney nickel (2.0 g. wet, washed twice with *p*H 7 buffer solution) was added to a mixture of 5-amino-4-(1-benzylhydrazino)-6-chloropyrimidine (200 mg.) in *p*H 7 buffer solution (25 ml.) and the resultant mixture refluxed for 2 hours. The mixture was filtered hot, the filtrate evaporated to dryness *in vacuo*, and the residue extracted with chloroform (2 × 10 ml.). Evaporation of the combined extracts and sublimation of the residue at 124° (0.1 mm.) gave a white solid; yield 75 mg., m.p. 130–132°. Recrystallization of this material from benzene gave a white solid, m.p. 136–137°. This material was identified as **5-amino-4-benzylaminopyrimidine** by elemental analysis and by comparison of its infrared and ultraviolet spectra with those of 5-amino-4-ethylaminopyrimidine[10]; spectral data: λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1, 290 (12.7); *p*H 7, 255 (9.1); 290 (8.3); *p*H 13, 255 (9.6), 290 (7.7); $\bar{\nu}$ in cm.$^{-1}$: 3380 and 3200 (NH); 3060 (aromatic CH); 2920 and 2880 (aliphatic CH); 1680 (NH); 1600, 1590, 1570 and 1510 (C=C, C=N); 1460 (aliphatic CH); 750 and 700 (monosubstituted phenyl).

Anal. Calcd. for $C_{11}H_{12}N_4$: C, 65.98; H, 6.04; N, 27.98. Found: C, 66.09; H, 6.04; N, 28.20.

B. Palladium.—A solution of 5-amino-4-(1-benzylhydrazino)-6-chloropyrimidine (1.00 g.) in 1:1 ethanol–water (100 ml.) containing magnesium oxide (1.00 g.) was hydrogenated over a 5% palladium-on-charcoal catalyst. After the steady uptake of one equivalent of hydrogen, there was a further, but much slower, absorption of hydrogen. The catalyst was removed by filtration and the residue was washed with ethanol (25 ml.). A 5% solution of sodium carbonate (50 ml.) was added to the combined wash and filtrate, and the whole evaporated to dryness. Extraction of the residue with chloroform (2 × 50 ml.) and evaporation of the combined extracts gave 550 mg. of material, m.p. 101° with softening from 95°. Sublimation of this sample at 105° (0.1 mm.) gave pure **5-amino-4-(1-benzylhydrazino)-pyrimidine** (**XXI**, R = $CH_2C_6H_5$); yield 510 mg. (59%), m.p. 100–101°; spectral data: λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1, 293 (shoulder) (8.3), 314 (9.3); *p*H 7–265 (7.6), 307 (8.4); *p*H 13, unstable; $\bar{\nu}$ in cm.$^{-1}$: 3410 and 3300 (NH); 3040 (aromatic CH); 1650 (NH); 1600, 1575, 1550 and 1490 (C=C, C=N); 1460 (aliphatic CH); 730 and 700 (monosubstituted phenyl).

Anal. Calcd. for $C_{11}H_{13}N_5$: C, 61.37; H, 6.09; N, 32.54. Found: C, 60.93; H, 6.08; N, 32.29.

1-Benzyl-5-chloro-1,2-dihydropyrimido[5,4-*e*]-*as*-triazine (**XXII**).—A solution of 5-amino-4-(1-benzylhydrazino)-6-chloropyrimidine (320 mg.) in formic acid (10 ml.) was refluxed for 4 hours, evaporated to a small volume *in vacuo*, and the residue heated to boiling in 2 *N* hydrochloric acid (40 ml.). The acidic solution was treated with Norit and the filtrate neutralized to *p*H 6 with concentrated ammonium hydroxide. The solid that deposited was collected by filtration, dissolved in acetone (10 ml.), a small amount of insoluble material removed by filtration, and the filtrate evaporated to dryness *in vacuo*. The solid was dried *in vacuo* over P_2O_5 at 56° for 3 hours; yield 150 mg. (48.5%), m.p. 182–183° dec.; spectral data: λ_{max} in mμ: *p*H 1, 334 (3.98); *p*H 7, 226 (11.2); 244 (9.33), 346 (2.78); *p*H 13, 248 (8.46), 280 (4.97); $\bar{\nu}$ in cm.$^{-1}$: 3265 (NH); 2940 (aliphatic CH); 1655 (C=N); 1600, 1575, 1550 and 1480 (C=C, C=N); 740 and 700 (monosubstituted phenyl).

Anal. Calcd. for $C_{12}H_{10}ClN_5$: C, 55.50; H, 3.85; N, 27.00; Cl, 13.67. Found: C, 55.06; H, 4.02; N, 27.01; Cl, 13.87.

1,2-Dihydro-1-methylpyrimido[5,4-*e*]-*as*-triazine (**XXIII**, R = CH_3).—A solution of 5-amino-4-(1-methylhydrazino)-pyrimidine (480 mg.) in formic acid (50 ml.) was refluxed for 0.5 hour and evaporated to dryness under reduced pressure. The reddish-brown residue was then heated on a water-bath under high vacuum until the color of the residue had changed to yellow; yield 580 mg. This material decomposes rapidly without melting above 175° and leaves a residue on combustion.

A portion (200 mg.) of this material was extracted with ether (3 × 100 ml.); the extracts were combined and evaporated to dryness in a stream of nitrogen to give a slightly brown solid; yield 100 mg., m.p. 202° dec. (when taken rapidly from 175°); spectral data: λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1, 239 (shoulder, 5.1), 331 (4.55); *p*H 7, 239 (shoulder), 335 (unstable); *p*H 13, unstable; $\bar{\nu}$ in cm.$^{-1}$: 3430 and 3220 (NH); 3040 (=CH); 2910 and 2840 (aliphatic CH); 1660, 1600 and 1500 (C=C, C=N).

Anal. Calcd. for $C_6H_7N_5$: C, 48.31; H, 4.73; N, 46.96. Found: C, 47.84; H, 4.90; N, 47.13.

1-Benzyl-1,2-dihydropyrimido[5,4-*e*]-*as*-triazine (**XXIII**, R = $CH_2C_6H_5$).—A solution of 5-amino-4-(1-benzylhydrazino)-pyrimidine (460 mg.) in 98–100% formic acid (25 ml.) was refluxed for 30 minutes, evaporated to dryness, and the residue dissolved in 0.5 *N* hydrochloric acid (15 ml.). The solution was neutralized with 1 *N* sodium hydroxide, and the oil that deposited extracted with ether (3 × 50 ml.). Evaporation of the ether and trituration of the residue with a small amount of methanol gave a light yellow solid; yield 220 mg. (46%).

A small sample of this material was recrystallized from benzene by the addition of Skellysolve C; spectral data: λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1, 335 (4.3); *p*H 7, 341 (3.9); *p*H 13, unstable; $\bar{\nu}$ in cm.$^{-1}$: 3200 (NH); 2950 (aliphatic CH); 1660 (C=N); 1600, 1590, 1570 and 1490 (C=C, C=N); 775 and 700 (monosubstituted phenyl).

Anal. Calcd. for $C_{12}H_{11}N_5$: C, 63.98; H, 4.92; N, 31.09. Found: C, 63.91; H, 4.91; N, 30.96.

[CONTRIBUTION FROM THE ORGANIC CHEMICAL RESEARCH SECTION, LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID CO., PEARL RIVER, N. Y.]

N,N′-Carbonyldiimidazole, a New Peptide Forming Reagent[1]

BY ROLF PAUL AND GEORGE W. ANDERSON

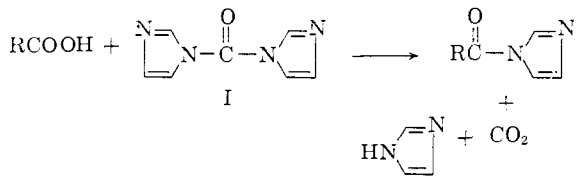
RECEIVED FEBRUARY 9, 1960

N,N′-Carbonyldiimidazole was shown to be a useful peptide forming reagent. Conditions were worked out for avoiding racemization in the formation of ethyl carbobenzoxyglycyl-L-phenylalanylglycinate, a sensitive case.

It has been demonstrated by Wieland and Schneider[2] that peptide derivatives can be synthesized through acylation of the imidazole ring of methyl N-benzoyl-L-histidinate followed by reaction with the appropriate amine. Their method, however, was not suitable for general use because of low yields. It occurred to us that a more direct agent for making acyl-imidazoles might be N,N′-carbonyldiimidazole. This would be a convenient reagent since the by-products, carbon dioxide and imidazole, are innocuous. The carbon dioxide evolution would provide a driving force for the reaction.

(1) Preliminary communication G. W. Anderson and R. Paul, THIS JOURNAL, **80**, 4423 (1958).

(2) T. Wieland and G. Schneider, *Ann.*, **580**, 159 (1953); see also M. Bergmann and L. Zervas, *Z. physiol. Chem.*, **175**, 145 (1928).



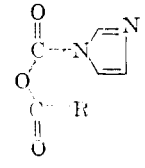
Staab[3] has shown that N,N'-carbonyldiimidazole (I) is reactive toward amines and alcohols forming ureas and carbonates. Extending this, we found that acids react to give acyl-imidazoles, and the reaction can be used in peptide synthesis. Ethyl carbobenzoxyglycyl-L-tyrosinate (II), for instance, was made in 95% yield by this method.

Difficulties were encountered in following the literature[3] preparation of the reagent. These were solved by preparing N,N'-carbonyldiimidazole from imidazole and phosgene in rigorously dried benzene. It was important to use a slight excess of imidazole to prevent contamination of the product by half-reacted phosgene. Likewise it was found important to remove all the imidazole hydrochloride from the reagent. Contaminated N,N'-carbonyldiimidazole gave poor yields in peptide synthesis and was deliquescent. The crude reagent was assayed for carbon dioxide on hydrolysis; the purity ranged from 91 to 100% and the melting point of the better material was 113–115°.

A typical peptide reaction was run by treating a solution of 0.010 mole of an acylamino acid in 10 ml. of tetrahydrofuran (THF) with 0.010 mole of reagent, adjusted for that quantity from the assay. The evolution of carbon dioxide was immediately observed. After an hour the desired amino acid or peptide ester was added in 0.010 molar quantity. A small amount of heat was evolved. The reaction has been worked up after 15 min., but longer standing is probably beneficial. The product was isolated by removing the solvent under vacuum followed by washing the residue with *N* acid, saturated bicarbonate and finally water.

It is critical to maintain absolute dryness during the reaction of the acid with the N,N'-carbonyldiimidazole since the reagent decomposes almost instantly on contact with water. The intermediate acylimidazole is stable toward hydrolysis for short periods of time.

To get a better picture of the course of the reaction, carbobenzoxyglycine was treated with the reagent and the carbobenzoxyglycylimidazole (III) was isolated and analyzed. It was treated further with ethyl L-tyrosinate to give an 83% yield of ethyl carbobenzoxyglycyl-L-tyrosinate. A possible mechanism for the acyl-imidazole formation would be an attack on the carbonyl carbon of the reagent by the oxygen of the acid or a carboxylate ion followed by elimination of an imidazole molecule to give



Models show that the amide nitrogen of the imidazole can be brought into contact with the carboxylate suggesting a simultaneous bond formation and carbon dioxide elimination. An alternate path is, of course, a simple displacement by a second molecule of imidazole.

It is possible to obtain peptides from acylimidazoles by reaction with an amino acid ester hydrochloride instead of the free base and even by using an aqueous solution of an amino acid salt. In the latter case, the yields are lower. Carbobenzoxyglycine, for example, was treated with the reagent in THF followed by a solution of sodium DL-phenylalaninate in water to give a 55% yield of carbobenzoxyglycyl-DL-phenylalanine (IV). A limitation to the last method is the low solubilities of some amino acid salts in water. Likewise a limitation on the use of amino acid ester hydrochlorides is their insolubilities in organic solvents.

The importance of using precise amounts of assayed N,N'-carbonyldiimidazole was overlooked at first since the examples studied gave reasonably good yields and little trouble was encountered in purifying them. It was assumed that the desired reactions went at a much faster rate than side reactions. As more reactions were studied it became evident, however, that while acylimidazole formation was relatively fast and complete, the reaction of the acylimidazole with an amine to form a peptide was about as fast as the reaction of amine with unreacted N,N'-carbonyldiimidazole to form ureas. Ureas being difficult to separate, it was best to avoid their formation through the use of precise amounts of assayed reagent. On using an excess of reagent in a preparation of ethyl carbobenzoxyglycyl-L-tyrosinate, the crude product had a poor melting point and was difficult to purify. By the time a reasonable melting point was achieved, the yield had dropped to 64% as compared to 95% obtained previously.

In another experiment to determine the best solvent for the reaction little difference was noted among six tried. The only one out of line was methylene chloride which gave a higher yield. This difference could be accounted for by the fact that the solvent was boiled off rather than removed at room temperature and the heating made the reaction go farther. To test this, *t*-butyl trifluoroacetylglycyl-L-prolinate (V) was obtained in 53% crude yield when prepared in the usual fashion. In another run, after the addition of the *t*-butyl L-prolinate, the reaction mixture was heated on a steam-bath for 1 hour, giving a higher crude yield, 61%. However, heating is not recommended as a general procedure since it increases side reactions.

In several other experiments (see Table I) the best conditions for the formation of one particular dipeptide were worked out. Mixing the acid and amine followed by the reagent gave no isolatable product. Removal of the reaction solvent at room temperature before work-up gave a slight (2%) improvement. The best yield was obtained in a run where the reaction of reagent with acid was permitted to go for at least 30 minutes at room temperature before the amine was added.

(3) H. A. Staab, *Ann.*, **609**, 75 (1957).

TABLE I

VARIATION OF REACTION CONDITIONS IN THE FORMATION OF Z·gly-tyr·OEt(L) (II)

Variation	Solvent	Recrystd. yield, %	M.p., °C.
Used excess Im_2CO	THF	63	125–128
Heated before addn. of H·tyr·OEt (L)	THF	59	127–129.5
Heated after addn. of H·tyr·OEt (L)	THF	70	127–128
None	THF	71	127–128
None	$(CH_3OCH_2)_2$	67	127–128
Boiled solvent off	CH_2Cl_2	78	126–127
None	Pyridine	74	126–127
None	DMF	66	126.5–128
None	$(EtO)_2POH$	73	126.5–128
Used HBr·H·tyr·OEt(L); boiled off solv.	CH_2Cl_2	80	126.5–127.5
Calcd. amt. of Im_2CO used here and below	CH_2Cl_2	90	124–125.5
Removed solv. before workup; 2 recrystn.	THF	86	124–126.5
Kept solvent; 2 recrystn.	THF	84	125–127
Ran at −15°; 4 recrystn.	DMF	79	123–127
Combined Z·gly·OH + H·tyr·OEt (L) then added Im_2CO	THF	Non-crystallizable oil	
30 min. for 1st step, 12 hr. 2nd, vac. off solv., 1 recrystn.	THF	95	127–128
30 min. for 1st step, 12 hr. 2nd, vac. off solv., 1 recrystn.	CH_2Cl_2	89	125–127

Racemization was investigated in the reaction of carbobenzoxyglycyl-L-phenylalanine and ethyl glycinate, a very sensitive case.[4] It was necessary to determine the purity of the phenylalanine derivative since commercial L-phenylalanine may contain as much as 5% DL-material. The sample used for the racemization study was carefully purified by fractional crystallization. It was checked for racemic content and shown to be pure L-isomer. In running the test on N,N′-carbonyldiimidazole at room temperature in THF, 5% racemic material was found in the tripeptide thus produced. However, if the reaction was run in DMF (dimethylformamide) at −10° less than 0.5% racemic material was detected.

The possibility of using other reagents similar to N,N′-carbonyldiimidazole was investigated. N,N′-Carbonyldibenzimidazole[5] was made and found to be less reactive than the imidazole compound. Several attempts to make N,N′-sulfonyldiimidazole failed. Table II gives yields for other peptides made with N,N′-carbonyldiimidazole and compares them to literature preparations. An attempt has been made to quote the higher and oftimes the highest literature yields.

The use of N,N′-carbonyldiimidazole in the synthesis of angiotensin is currently under investigation.

Acknowledgments.—We thank Mr. L. Brancone and staff for analyses and Mr. W. Fulmor and staff for optical rotations.

Experimental

Melting Points.—All melting points were run on a calibrated Fisher–Johns block unless otherwise indicated.

Solvents—All solvents used were dried; THF was distilled from calcium hydride; DMF was dried by azeotroping with benzene and storing over anhydrous magnesium sulfate.[6]

N,N′-Carbonyldiimidazole[3] (I).—All equipment used in this reaction was dried in an oven at 115°. A 2.2-l. portion of benzene was placed on 5–10 g. of calcium hydride and 2.0 l. was distilled into a 3-liter, 3-necked flask containing 112 g. (1.65 moles) of imidazole and equipped with a stirrer and drying tube. When the benzene had been distilled over, the condenser was replaced by a gas inlet tube and the mixture heated and stirred to obtain a clear solution. A 38.6-g. (0.40 mole) quantity of phosgene was collected as a liquid *via* a Dry Ice condenser and distilled, by warming by hand, into the reaction mixture. A precipitate formed at this point. After all the phosgene was distilled, the system was flushed with dry nitrogen for a few minutes, cooled to 30° and let stand until the liquid phase was clear (from 1 to 24 hr.), then warmed to 50° and filtered. Most of the benzene was removed under reduced pressure at 60°. The resulting slush was cooled and quickly filtered. Overnight drying under reduced pressure gave 50.4 g. of N,N′-carbonyldiimidazole, a 77% yield. The material, on a capillary melting point, shrank at 111°, started to liquify at 113°, was a cloudy liquid at 115° and all clear at 117° (lit.[3] m.p. 115.5–116°). The reagent was assayed for carbon dioxide on hydrolysis. A calculated CO_2 value of 27.2% compared with the 26.7 ± 0.5% CO_2 found by analysis showed the final product to be 98 ± 2% pure. A silver nitrate test on an acidified sample in water solution was negative. On several runs yields of 75–82% and purities from 91 to 99.5% were obtained.

Ethyl Carbobenzoxyglycyl-L-tyrosinate (II).—To a solution of 2.09 g. (0.010 mole) of carbobenzoxyglycine[7] in 10 ml. of dry THF was added 1.62 g. (0.010 mole)[8] of N,N′-carbonyldiimidazole. One-half hour later, 2.09 g. (0.010 mole) of ethyl L-tyrosinate[9] was added. After standing overnight, the THF was removed by an air stream and 50 ml. of 1 *N* hydrochloric acid was added. Cooling of the solution gave a solid. This was washed with water, triturated with 20 ml. of 5% sodium bicarbonate solution, filtered and again washed with water. The product was dried in a steam cabinet and yielded 3.93 g. (98%), having a melting point of 125.5–127°. Recrystallization from 50% ethanol gave 3.79 g. (95%) of material with a melting point of 127–128°. The optical rotation, $[\alpha]^{25}D$ + 18.2 ± 1.0° (*c* 5, abs. ethanol), and the melting point compare favorably with lit. values[10] of $[\alpha]^{24}D$ + 19.3 ± 0.1°, m.p. 125–126.5°, and yield 68%.

By waiting 15 min. before the addition of the ethyl L-tyrosinate an 83% yield of recrystallized material, m.p. 126–127°, was obtained.

N-(Carbobenzoxyglycyl)-imidazole (III).—N,N′-Carbonyldiimidazole (1.91 g., 0.011 mole, 93% pure) and 2.09 g. (0.010 mole) of carbobenzoxyglycine were dissolved in 10 ml. of dry THF. After the effervescence stopped the solution was diluted with 30 ml. of ether. The product crystallized and was collected by filtration. It was washed with ether and dried in a desiccator to give 1.92 g., m.p. 115–118°. The mother liquors yielded an additional 0.16 g., m.p. 117–118°, giving a total yield of 80%. On recrystallization from THF–ether, 1.57 g. (60%), m.p. 119–120°, was obtained.

Anal. Calcd. for $C_{13}H_{13}N_3O_3$: C, 60.22; H, 5.05; N, 16.21. Found: C, 60.41; H, 5.31; N, 16.04.

The material partially decomposed on standing in the open overnight as determined by a drop in melting point to 80–88°. A vialed sample decomposed slightly over the period of a month to 113–118°. Perhaps this was due to the moisture in the air of the vial.

A 0.259-g. (0.001 mole) sample of Z·gly·Im was treated with 0.209 g. (0.001 mole) of ethyl L-tyrosinate in 2 ml. of THF. After standing overnight water was added giving a basic solution and an oil. The oil was washed with water, *N* acid and water. Drying in an oven gave 0.362 g. (90%) of material, m.p. 121–126°. Recrystallization gave 0.331 g. (83% yield), m.p. 123–126°, of ethyl carbobenzoxyglycyl-L-tyrosinate.

(4) G. W. Anderson and F. M. Callahan, THIS JOURNAL, **80**, 2902 (1958).

(5) H. A. Staab and G. Seel, *Ann.*, **612**, 187 (1958).

(6) A. B. Thomas and E. G. Rochow, THIS JOURNAL, **79**, 1843 (1957).

(7) M. Bergmann and L. Zervas, *Ber.*, **65**, 1192 (1932).

(8) In some of the earlier experiments the necessity of having each batch of N,N,-carbonyldiimidazole assayed was not recognized.

(9) E. Fischer, *Ber.*, **34**, 433 (1901).

(10) J. R. Vaughan, Jr., and R. L. Osato, THIS JOURNAL, **74**, 676 (1952).

TABLE II

PEPTIDES DERIVATIVES PREPARED USING N,N′-CARBONYLDIIMIDAZOLE[w]

No.	Product	Pure yield, %	M.p., °C.	Recrystn. solvent	$[\alpha]^{25}D$ (c)	Lit. yield, %	Lit. m.p., °C.
1	Z·gly-tyr·OEt(L)[a]	95	127–128	50% ethanol	+18.2 ± 1.0° (abs. EtOH)	68[b]	125–126.5
2	B·phe-gly·OEt(L)[c]	78	88–89.5	Pet. ether	−4.2 ± 1.2 (EtOH)	81[d]	86–87
3	Z·gly-phe·OEt(DL)[e]	80	90–91	Benzene–pet. ether		86[f]	91–92
4	Z·gly-phe·OEt(DL)[g]	82	91–92	Benzene–pet. ether		86[f]	91–92
5	Z·gly-phe·OH(DL)	55	162.5–163.5	50% ethanol		63[b]	160–162
6	Z·gly-phe·OH(L)	40	126.5–127.5	Water	+40.7 ± 1.7 (abs. EtOH)		127.5[h]
7	Phth·phe-gly-gly·OEt(DL)[i]	56	163–164.5	Benzene–pet. ether		67[b]	162–163
8	Z·ala-gly·OEt(L)	65	98–99	EtOAc–pet. ether	−21.7 ± 0.5 (abs. EtOH)	77[j]	100
9	Z·gly-leu·OH(L)[k]	68	103–104	EtOAc–pet. ether	−18.2 ± 0.5 (1 *N* NaOH)	62[l]	104
10	Z·gly-gly·OEt[m]	60	82.5–83	50% ethanol		83	86–87[n]
11	TFA·gly-pro·O*t*Bu(L)[o]	51	90–91	Methylcyclohexane	−83 (EtOH)	69[p]	89–90
12	Z·phe-tyr·OEt(L,L)[q]	57	158–160	50% ethanol	−9.2 ± 0.5 (EtOH)	46[b]	159–160[r]
13	Z·asp(NH₂)-gly·OCH₂C₆H₅(L)[s]	35	177–179	Water		50[t]	181–183
14	Z·ileu-his OMe(L,L)	27	184	Methanol-water	+1.8 (DMF)	50[u]	186–189
15	Z·gly-phe-glyOEt(L)[w]	87	119.9–120.3	Abs. ethanol	−12.2 ± 1.25 (EtOH)	96[v]	120–120.5

[a] Z = Carbobenzoxy. [b] Ref. 10; mixed anhydride method. [c] B = *t*-butyloxycarbonyl. [d] G. W. Anderson and A. C. McGregor, THIS JOURNAL, **79**, 6183 (1957). Recrystallized to 35% yield, m.p. 89.5–90°, from ethyl acetate–petr. ether; pyrophosphite method. [e] Free H·phe·OEt(DL) used. [f] Ref. 12; mixed anhydride method. [g] Using HBr·H·phe·OEt-(DL)[11] directly. [h] Ref. 4. [i] Phth = phthaloyl; from Phth·phe·OH and H·gly-gly·OEt. [j] M. Bergmann, L. Zervas, J. S. Fruton, F. Schneider and H. Schleich, *J. Biol. Chem.*, **109**, 325 (1935); acid chloride method. [k] The ethyl ester is an oil and was saponified; the yield is the over-all yield. [l] H. S. Goldschmidt and H. Lautenschlager, *Ann.*, **580**, 68 (1953); phosphorus trichloride method, followed by saponification of the ethyl ester thus formed. [m] Using HCl·H·gly·OEt directly. [n] Ref. 14a using the pyrophosphite method. O. Süs, *Ann.*, **572**, 104 (1951), reports m.p. 82.5–83° and a 25% yield using the phosphorus trichloride method. [o] TFA = trifluoroacetyl. [p] Ref. 13; pyrophosphite method, crude yield. [q] Amorphous. [r] J. S. Fruton and M. Bergmann, *J. Biol. Chem.*, **145**, 262 (1942), using Z·phe·Cl obtain 46%, m.p. 162°. [s] Amine used as benzenesulfonate; product was recrystallized three times. [t] H. K. Miller and H. Waelsch, *Arch. Biochem. Biophys.*, **35**, 176 (1952); diethyl chlorophosphite method. [u] H. Schwarz, F. M. Bumpus and I. H. Page, THIS JOURNAL, **79**, 5697 (1957); mixed anhydride method. [v] Ref. 4; pyrophosphite method. [w] The general procedure used for the synthesis of Z·gly-tyr·OEt(L) was used in each case.

Ethyl Carbobenzoxyglycyl-DL-phenylalaninate.—Ethyl DL-phenylalaninate was prepared from 2.75 g. (0.010 mole) of ethyl DL-phenylalaninate hydrobromide[11] on treatment with an excess of triethylamine in THF followed by filtration and concentration. A solution of 2.09 g. (0.010 mole) of carbobenzoxyglycine in 10 ml. of dry THF was treated with 1.62 g. (0.010 mole) of N,N′-carbonyldiimidazole. When the effervescence ceased, the previously prepared ethyl DL-phenylalaninate was added. After 15–30 minutes, the mixture was air-dried and 50 ml. of 1 *N* hydrochloric acid added. On cooling, a solid formed. This was collected, triturated first with water, then with a 5% sodium bicarbonate solution, and again with water. Drying gave 3.20 g. (83% yield) of ethyl carbobenzoxyglycyl-DL-phenylalaninate with a melting point of 90–91°. The material was recrystallized from benzine–petroleum ether, resulting in 3.10 g. (80% yield). The product had a melting point of 90–91°, lit. value[12] 91–92°. This experiment was repeated except that the ethyl DL-phenylalaninate hydrobromide was added directly to the reaction mixture without first removing the HBr with triethylamine. An 82% yield of a product having a melting point of 91–92° was obtained by this method.

Carbobenzoxyglycyl-DL-phenylalanine (IV).—A solution of 2.09 g. (0.010 mole) of carbobenzoxyglycine in 10 ml. of dried THF was treated with 1.62 g. (0.010 mole) of N,N′-carbonyldiimidazole. When the effervescence stopped, a solution of 1.64 g. (0.010 mole) of DL-phenylalanine in 10 ml. of 1 *N* sodium hydroxide was added. After 15–30 minutes, 50 ml. of 1 *N* hydrochloric acid was added. The oily liquid thus formed crystallized in a few minutes. The product, carbobenzoxyglycyl-DL-phenylalanine, was collected, washed with water and dried to give 2.51 g. (70% yield) of material having a melting point of 158–160°. The product was recrystallized from 35 ml. of 50% ethanol giving 1.97 g. (55% yield) of the dipeptide with a melting point of 162.5–163.5°, lit. value[10] 160–162°.

The above experiment was repeated using L-phenylalanine in place of DL-phenylalanine. A 40% yield of material was obtained which had a melting point of 126.5–127.5° and an optical rotation $[\alpha]^{25}D$ + 40.7 ± 1.7° (*c* 2.9, absolute ethanol); the corresponding lit. values[4] were m.p. 127.5° and $[\alpha]^{25}D$ + 38.8 ± 0.5° (*c* 5, absolute ethanol).

***t*-Butyl Trifluoroacetylglycyl-L-prolinate (V).**—The solution resulting from the reaction of 1.71 g. (0.01 mole) of trifluoroacetylglycine[13] in 10 ml. of THF with 1.74 g. (0.01 mole, 93% pure) of N,N′-carbonyldiimidazole was permitted to stand for an hour. Then 1.71 g. of *t*-butyl L-prolinate[14] was added. After standing overnight, the solvent was removed under vacuum and the residual yellow oil triturated with 35 ml. of *N* acid. The product crystallized readily. The colorless solid was collected, triturated with water and dried, giving 1.70 g. (53%), m.p. 89–90°. One recrystallization from methylcyclohexane gave 1.65 g. (51% yield) of material, m.p. 90–91°.

In a second run the reaction was warmed on a steam-bath for 1 hr. after the addition of the amine instead of leaving it overnight at room temperature. In this case, the crude yield was 1.97 g. or 61%, m.p. 89–90°, $[\alpha]^{25}D$ − 83° (*c* 2, ethanol); lit.[14] m.p. 89–90°.

Racemization Studies with N,N′-Carbonyldiimidazole.—The reaction of carbobenzoxyglycyl-L-phenylalanine with ethyl glycinate is very sensitive to racemization.[4,15] It was shown[4,15] that using tetraethylpyrophosphite as the peptide-forming agent gave no racemization. To test the purity of the starting dipeptide acid the reaction was run using tetraethylpyrophosphite and no racemic material

(11) Made from DL-phenylalanine, ethanol and dry hydrogen bromide; m.p. 131–132°. *Anal.* Calcd. for $C_{11}H_{16}NO_2Br$: C, 48.19; H, 5.88; N, 5.11. Found: C, 48.32; H, 6.01; N, 5.39.

(12) J. R. Vaughan, Jr., and R. L. Osato, THIS JOURNAL, **73**, 5553 (1951).

(13) F. Weygand and E. Leising, *Ber.*, **87**, 248 (1954).

(14) G. W. Anderson and F. M. Callahan, THIS JOURNAL, **82**, 3359 (1960).

(15) (a) G. W. Anderson and R. W. Young, *ibid.*, **74**, 5307 (1952); (b) G. W. Anderson, J. Blodinger and A. D. Welcher, *ibid.*, **74**, 5309 (1952); (c) J. R. Vaughan, Jr., *ibid.*, **74**, 6137 (1952).

was found. Proceeding from there, a solution of 3.56 g. (0.010 mole) of carbobenzoxyglycyl-L-phenylalanine [having a melting point of 127.5–128.0°, and an optical rotation $[\alpha]^{25}D$ + 38.2° (*c* 5, abs. ethanol) compared with values[4] of m.p. 127.5° and $[\alpha]^{24}D$ + 38.8 ± 0.5° (*c* 5, abs. ethanol)] in 10 ml. of dried (calcium hydride) dimethylformamide was cooled to −10° and 1.65 g. (0.010 mole based on 98% purity) of N,N′-carbonyldiimidazole was added. When the slow effervescence stopped, 1.03 g. (0.010 mole) of freshly-distilled ethyl glycinate was added. The reaction solution was allowed to warm to room temperature and permitted to stand 15–30 min. Then 50 ml. of 1 *N* hydrochloric acid was added. When the oily liquid thus formed solidified, it was washed with 20 ml. of a 5% sodium bicarbonate solution and with water. On drying, 4.22 g. (96% yield) with a melting point of 115.5–117.0° was obtained. The product was dissolved in 210 ml. of absolute ethanol to give a 2% solution. After cooling to 0°, the solution was seeded with a crystal of ethyl carbobenzoxyglycyl-DL-phenylalanylglycinate. Fractions were cut as follows:

No.	Time, hr.	Wt., g.		M.p., °C.
1	3	0.0091		120.0–133.5
2	6	.0097		119.0–128.5
3	10	.0214		118.5–119.5
4	24	2.3201		119.8–120.1
	Concd.	1.4787		119.9–120.3
	Residue	0.3271		
	DL-Isomer	0.0188	0.45%	
	L-Isomer	3.8202	87%	$[\alpha]^{25}D$ −12.2 ± 1.25° (*c* 2, EtOH)
	Residue	0.3271		
	Material balance	4.1661		

Since the melting point of pure ethyl carbobenzoxyglycyl-DL-phenylalanylglycinate was reported[4] to be 132–133° and has been found in other reactions by us to be 133.0–133.5°, the percentage of DL-tripeptide is estimated from the melting points to be much less than 0.5%.

In other reactions, run in the same way, varying one or two factors, more racemization was found. Running the reaction in THF at room temperature gave 5% DL-tripeptide. Using ethyl glycinate hydrochloride at room temperature in THF gave 8% DL-tripeptide. Running the reaction in dimethylformamide at 0–5° gave 1.3% racemization.

In these studies, the melting point of the carbobenzoxyglycyl-L-phenylalanine used was very important since commercial L-phenylalanine may contain several per cent. of the DL-isomer.

Ethyl Carbobenzoxyglycyl-DL-phenylalaninate using N,N′-Carbonyldibenzimidazole.—A solution of 2.09 g. (0.010 mole) of carbobenzoxyglycine in 10 ml. of dry THF was treated with 2.62 g. (0.010 mole) of N,N′-carbonyldibenzimidazole.[5] When no effervescence was noted, and the reagent only partially dissolved, another 10 ml. of THF was added. Again nothing happened and the mixture was heated under reflux for 10 min. A solution formed and 1.93 g. (0.010 mole) of ethyl DL-phenylalaninate (freshly distilled) was added. After heating for 15–30 min. on a steam-bath, most of the solvent boiled off. A 50-ml. quantity of 1 *N* hydrochloric acid was then added. Cooling and scratching gave a solid product. This was washed first with water, then with 20 ml. of 5% sodium bicarbonate solution and finally with water again. The crude product weighed 3.48 g. (77% yield) and had a melting point range of 83.5–88.0°. Recrystallization from 20 ml. of ethyl acetate and 40 ml. of petroleum ether resulted in 2.75 g. of compound with a melting range of 85–88°. This material was recrystallized again, this time from 20 ml. of benzene and 40 ml. of petroleum ether, giving 2.63 g. (69% yield) of ethyl carbobenzoxyglycyl-DL-phenylalaninate with a melting point of 89.5–91.0° as compared to a previously cited melting point of 90–91°. Because of the poorer yield and the more rigorous conditions necessary for a reaction, N,N′-carbonyldibenzimidazole is considered inferior to N,N′-carbonyldiimidazole.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, YALE UNIVERSITY]

Some Reactions of N-Ethylmaleimide[1]

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RECEIVED MARCH 7, 1960

The reactions of N-ethylmaleimide (NEM) with the amino group of peptides, with imidazole and with cysteine have been investigated. With the first two classes of compound, an N-acylation reaction appears to occur, followed in the case of imidazole by a catalytic polymerization of NEM. With cysteine, reaction proceeds through addition of the thiol to the olefinic bond of NEM; in alkaline solution, the cysteine adduct undergoes an intramolecular transamidation reaction to form a thiazane derivative.

During the course of studies on the action of cysteine-activated cathepsin C[4] on glycyl-L-histidinamide at *p*H 7.4, N-ethylmaleimide (NEM) was used to facilitate chromatographic examination of the composition of the incubation mixture. The reaction of NEM with sulfhydryl compounds[5,6] had been used by Hanes, *et al.*,[7] to stabilize glutathione and other sulfhydryl peptides in paper chromatography. When the NEM-treated incubation mixtures of cathepsin C and glycyl-L-histidinamide were chromatographed, a number of Pauly-reactive components of widely different R_f values were observed.[8] Subsequent control experiments demonstrated, however, that the new products (other than the expected hydrolytic product, glycyl-L-histidine, or the unchanged dipeptide amide) arose in incubation mixtures to which no enzyme had been added. Further investigations showed that one of the new Pauly-positive products was noted only when NEM had been used before paper chromatography, and led to the recognition that NEM is not specific toward sulfhydryl compounds, as had previously been supposed. In the present communication we report some reactions of NEM with imidazole and its derivatives, with the α-amino group of

(1) This investigation was supported by grants from the National Science Foundation (G-7451) and the United States Public Health Service (RG-6452).

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