Anal. Calcd: C, 69.0; H, 8.7. Found: C, 68.8; H, 8.6.

Methyl 3α,7α-Diacetoxy-11β-bromo-12-oxocholanate (7).—11α,12α-Epoxide 5 (400 mg) in acetone (40 ml) was subjected to the action of 48% HBr (1 ml). The crude bromohydrin 6 (400 mg) was recrystallized from methanol to give a crystalline material: mp 176°; nmr ($CDCl_3$) δ 1.00 (s, 3, 18-CH_3), 1.18 (s, 3, 19-CH_3), 1.88 and 1.91 (-$OCOCH_3$), 3.50 (s, 3, 24-OCH_3), 4.21 (m, 1, HCBr).

A 200-mg portion of the unpurified bromohydrin was oxidized (CrO_3).[9] The crude product was recrystallized from methanol to give 160 mg of 11β-bromo ketone **7**: mp 190–191°; $[\alpha]_D$ ($CHCl_3$) +15.9°; ir 1738 (C=O), 1710 (C=O), 1245 (OC), 660 cm^{-1} (axial CBr); uv (ethanol) 310 nm (ε 110); nmr ($CDCl_3$) δ 1.37 (s, 18- and 19-CH_3), 2.02 (s, 3 and 7 -$OCOCH_3$), 3.66 (s, 3, 24-OCH_3), 4.42 (m, 1, HCBr); nmr (CCl_4) δ 1.37 (s, 18- and 19-CH_3), 1.90 and 1.98 ($OCOCH_3$), 3.60 (s, 3, 24-OCH_3), 4.42 (m, 1, HCBr).

Anal. Calcd: C, 59.1; H, 7.3; Br, 13.6. Found: C, 59.4; H, 7.3; Br, 13.3.

Epimerization of 11β-Bromo Ketone 7 to 11α-Bromo Ketone 2 with HBr.—To a solution of 7 (250 mg) in acetic acid (10 ml) 10% HBr in acetic acid (3 ml) was added. The reaction mixture was kept at room temperature for 2 days. Water was added and the precipitate was extracted with chloroform. The solvent was removed and diazomethane in ether was added to the residue. Recrystallization of the solid material from isopropyl alcohol afforded pure crystals identical in all respects with 11α-bromo ketone **2**. The 11β-bromo epimer was detected in the mother liquor.

An Unsuccessful Attempt to Epimerize 11β-Bromo Ketone 7 with BF_3.—To a solution of 7 (200 mg) in acetic acid (10 ml) 10 drops of BF_3 etherate were added and the solution was kept at room temperature for 5 days. The isolated material was identical with **7**; the 11α-bromo epimer was by no means present.

The Use of IBr as a Brominating Agent. A. With HBr as Catalyst.—To a solution of 1 (1 g) in acetic acid (30 ml), IBr (0.22 ml of bromine and 0.92 g of iodine) in acetic acid and 5 drops of 10% HBr in acetic acid were added. The reaction mixture was kept at room temperature for 7 days. The material which was isolated after the usual work-up proved to be identical with 1, mp 181°; the nmr spectrum was consistent with that previously reported;[12] for CD in ethanol, see Figure 1; for mass spectrum, see Table I.

B. With BF_3 as Catalyst.—The procedure was the same as above except that BF_3 etherate (5 drops) was used instead of HBr. The reaction mixture was kept at room temperature for 2 days and for 3 additional days at 35–40°. The only compound isolated was 3α,7α-diacetoxycholanic acid, nmr ($CDCl_3$) δ 8.75 (1, -COOH). The peak disappeared on addition of D_2O. Reaction with diazomethane gave 1. Note: the 12-keto group in **1**, **2**, and **7** was found to be unreactive to diazomethane.

Registry No.—**1**, 28535-81-1; **2**, 40488-36-6; **3**, 3749-87-9; **5**, 40488-38-8; **6**, 40488-39-9; **7**, 40488-40-2; borontrifluoride etherate, 109-63-7.

(12) Jeol, "High Resolution NMR Spectra," Sadtler Research Laboratories, Inc., 1967.

Conformations of Substituted Arylureas in Solution

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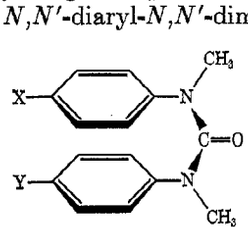
The conformation of *N,N'*-diarylureas in solution is investigated to obtain information about "stacking" interactions between aromatic rings. The only isomer which appears to exist has both aromatic rings in an anti relationship to the oxygen of the urea. Analysis of nmr and uv spectra suggests that there is a charge transfer interaction between the two rings, especially when they are substituted with electron-withdrawing and electron-donating groups.

During the past several years, a number of investigations[4] have suggested that, in aqueous solutions, parallel stacking of purine and pyrimidine bases is a major stabilizing force in oligo- and polynucleotides and in the binding of smaller aromatic compounds to nucleic acids. However, little is known about the specific factors which are responsible for this stacking. Theoretical studies have stressed the possible importance of dipole–dipole, dipole-induced dipole, London dispersion, and monopole–monopole interactions.

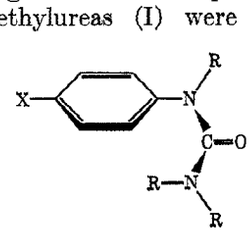
The crystal structure of *N,N'*-diethyl-*N,N'*-diphenylurea[5] has been shown to contain two phenyl rings aligned parallel to each other with their faces partially overlapping as shown in Figure 1. The preference for the conformation with the two bulky phenyl groups anti to the oxygen but in a "stacked" position near one another suggests that the same forces may be at work here.

In order to determine the conformation of diphenylureas in solution and to study the nature of the forces leading to the stacking of aromatic rings, we have studied the nuclear magnetic resonance and ultraviolet spectra of several substituted diphenylureas along with appropriate model compounds.

In order to establish the relative positions of the two phenyl rings, the proton magnetic resonance spectra of the *N,N'*-diaryl-*N,N'*-dimethylureas (I) were compared to those of the corresponding *N*-aryl-*N,N',N'*-trialkylureas (II). The results are shown in Table I.



Ia, X = Y = H
b, X = Y = = OCH_3
c, X = Y = NO_2
d, X = NO_2; Y = OCH_3



IIa, X = H; R = CH_3
b, X = OCH_3; R = CH_2CH_3
c, X = NO_2; R = CH_2CH_3

As may be seen, the aromatic protons of the diphenyl- (Ia) and dianisylureas (Ib) are uniformly shifted upfield in the presence of an aryl ring on the opposing nitrogen. This result suggests that the two rings are located near each other and are oriented so that the protons of each lie above the aromatic ring of the other so that a ring current induced upfield shift results.

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(3) Postdoctoral Fellow at the University of California, San Diego, 1972–present.

(4) C. E. Bugg, J. M. Thomas, M. Sundaralingam, and S. T. Rao, *Biopolymers*, **10**, 175 (1971), and references cited therein.

(5) P. Ganis, G. Avitabile, E. Benedetti, C. Pedone, and M. Goodman, *Proc. Nat. Acad. Sci.*, **67**, 426 (1970).

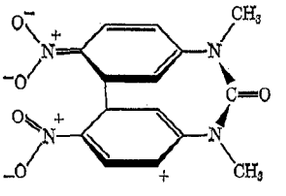
TABLE I

CHEMICAL SHIFTS[a] OF AROMATIC PROTONS IN *N*-ARYL-*N'*-METHYLUREAS AND *N,N'*-DIARYLUREAS

	N-Aryl-*N,N',N'*-trialkylurea (II)		*N,N'*-Diaryl-*N,N'*-dimethylurea (I)	
Substituent	Proton ortho to substituent	Proton meta to substituent	Proton ortho to substituent	Proton meta to substituent
H	7.09	7.32	6.79	7.02
			$\Delta\delta$ −0.30	$\Delta\delta$ −0.30
NO_2	8.11	6.93	8.02	7.07
			$\Delta\delta$ −0.09	$\Delta\delta$ +0.14
OCH_3	6.82	6.95	6.56	6.65
			$\Delta\delta$ −0.26	$\Delta\delta$ −0.30

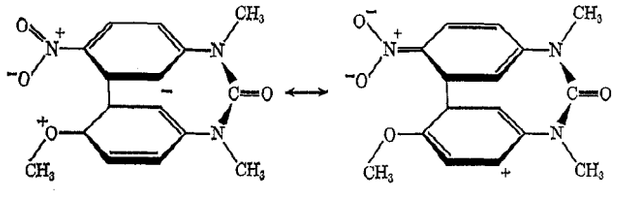
[a] In parts per million.

In contrast, the dinitrophenylurea Ic shows a smaller upfield shift for the proton ortho to the nitro group and a downfield shift for the meta protons. This result may be explained by interring electron withdrawal, which can be represented by resonance structures of the form



The importance of resonance of this type for the dianisylurea Ib is diminished by the fact that the two rings are already electron rich because of the acetamido-like substitution.

The nmr spectrum of the mixed urea clearly indicates a charge transfer effect in the interaction of the two aromatic rings. As may be seen in Table II, the protons of the nitrophenyl ring are shifted downfield and those of the anisyl ring are shifted upfield. This may be represented as resonance structures.



The lack of substantial solvent effect upon the chemical shifts is indicative of the relatively small contributions of the charge transfer to the ground state. The crucial mixed urea, Id, was studied over the concentrations 4.5–0.09%, a factor of 50, and showed no significant variation in any chemical shift. This experiment effectively rules out the possibility that the chemical shift effects result from aggregates such as dimers or trimers.

In order to establish whether the chemical shifts reported in Tables I and II represent the spectra of single conformers or the time-averaged spectrum of several conformers, the temperature dependence of the spectra were studied. Over the temperature range −30 to 55°, neither a change in coupling constants nor chemical shifts were observed in the spectra of Ia, Ib, Ic, or Id. These results indicate that either there is very strong preponderance of the syn isomer or that the equilibrium is temperature independent, which we consider to be very unlikely since the anti conformer would be very much less restricted than the syn conformer. Furthermore, at the lower temperatures, the spectra show no indications of splitting into bands for the two isomers, *i.e.*, there is no broadening of the signals.

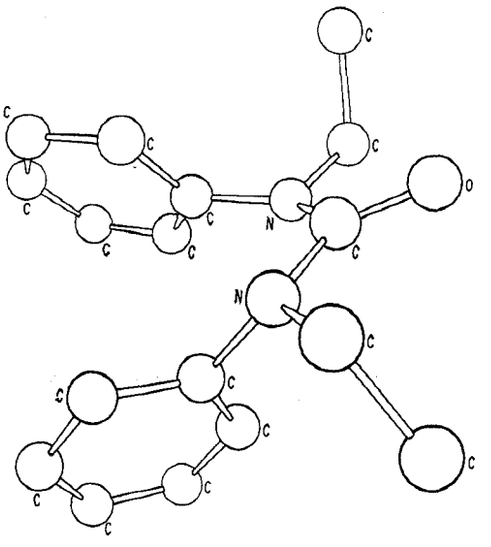


Figure 1.—Conformation of *N,N'*-diphenyl-*N,N'*-diethylurea emphasizing the relation of the two aromatic rings in space.

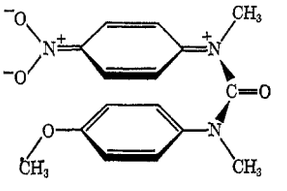
TABLE II

CHEMICAL SHIFTS[a] OF AROMATIC PROTONS OF *N*-ANISYL-*N'*-(*p*-NITROPHENYL)-*N,N'*-DIMETHYLUREA

Compd	Protons ortho to NO_2	Protons meta to NO_2	Protons ortho to OCH_3	Protons meta to OCH_3	Solvent
Ic	8.02	7.07			
Ib			6.56	6.65	$DCCl_3$
Id	7.95	6.94	6.61	6.77	
	$\Delta\delta$ −0.07	−0.13	+0.05	+0.12	
Ic	8.07	7.34			
Ib			6.59	6.68	O ‖ CD_3CCD_3
Id	8.02	7.16	6.72	7.00	
	$\Delta\delta$ −0.05	−0.18	+0.13	+0.32	
Ic	7.91	7.11			
Ib			6.57	6.68	CD_3CN
Id	7.93	7.00	6.66	6.89	
	$\Delta\delta$ −0.02	−0.11	+0.09	+0.21	
Ic	7.97	7.25			
Ib			6.64	6.73	O ↑ CD_3SCD_3
Id	7.97	7.09	6.73	7.00	
	$\Delta\delta$ 0.00	−0.16	+0.09	+0.27	

[a] In parts per million.

In order further to study the interaction of the two aromatic rings, we compared the ultraviolet spectra of the dinitrophenylurea Ib and the nitrophenyl-anisylurea Ic. The bands studied were in the region of 325–365 nm, and can be attributed to an intraring charge transfer of the nitrophenyl, the excited state of which can be represented as



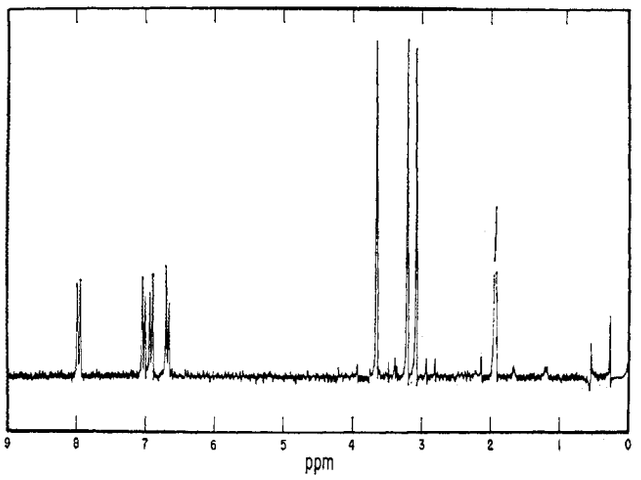


Figure 2.—Nuclear magnetic spectrum at 220 MHz in parts per million for *N,N'*-dimethyl-*N*-*p*-nitrophenyl-*N'*-*p*-methoxyphenylurea in CD_3CN at 25°.

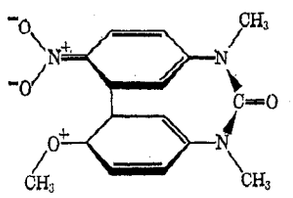
The spectra of the dianisylurea Ia are uninterpretable since λ_{max} lies under the solvent cutoff.

The results of these studies in different solvents are shown in Table III. The longer wavelength of the

TABLE III
ULTRAVIOLET SPECTRA OF DIARYLUREAS AT 25°

Solvent	*N,N'*-Di(*p*-nitrophenyl)-*N,N'*-dimethylurea (Ic)		*N*-(*p*-nitrophenyl)-*N'*-(*p*-anisyl)-*N,N'* dimethylurea (Id)	
	λ_{max}, nm	ϵ	λ_{max}, nm	ϵ
Chloroform	325	17,864	347	10,683
Acetone	337	17,600	355	12,740
Acetonitrile	333	17,364	354	12,011
DMSO	350	17,490	365	12,824

mixed urea can be attributed to stabilization of the excited state by participation of resonance structures of the form



Since the mixed urea has greater dispersal of charge in the excited state owing to this resonance participation, its λ_{max} is less affected by increased solvent polarity.

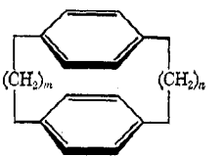
The temperature dependence of the ultraviolet spectra was also studied and the results are shown in Table IV. As stated earlier, lowering the temperature favors

TABLE IV
TEMPERATURE DEPENDENCE OF THE UV SPECTRA OF DIARYLUREAS IN ACETONITRILE

Temp, °C	*N,N'*-Di(*p*-nitrophenyl)-*N,N'*-dimethylurea (Ic)		*N*-(*p*-Nitrophenyl)-*N'*-(*p*-anisyl)-*N,N'* dimethylurea (Id)	
	λ_{max}, nm	$\epsilon/2$	λ_{max}, nm	ϵ
2	335	9137	355	12,632
25	333	8682	354	12,011
35	330	8619	353	11,932
50	328	8462	350	11,263

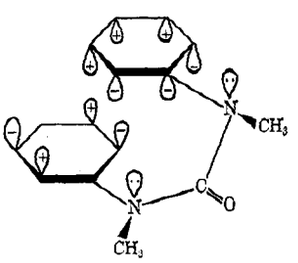
the more restricted forms of the molecule in which interaction between the rings is increased. Both spectra show increases in extinction coefficients with lowered temperature (1379 for the mixed urea and 676 for the dinitrourea when normalized for the presence of two chromophores). These changes depend upon the mixing of the charge transfer and ground states of the chromophores, and since this mixing is more affected by the anisyl ring than the nitro ring, the increased overlap resulting from closer contact is more evident in the mixed urea Id than the di(nitrophenyl) urea Ic.[6]

The study of compounds such as paracyclophanes[7] has shown that their spectral properties depend upon



the size of the macrocyclic system. It has been found that the λ_{max} increases with decrease in the distance between benzene rings. This effect is attributed to overlap of the two benzene rings. The nearly parallel changes in λ_{max} with temperature of the two ureas suggests that energy effects in both the ground and excited states are important and contribute to this value.

None of the data obtained by us offers evidence about the hybridization of the urea nitrogen atoms or the location of the rings other than that on the average they are close and in parallel positions. The similarities between the X-ray crystallographic structure and solution conformation are striking and lead us to expect the hybridization of the nitrogens and relative positions of the rings also to be similar. Theory suggests that the conformation in which the two aromatic rings are face to face in a mirror image relationship should not be favored. In the staggered relationship



shown in the X-ray structure, the overlap of HOMO of one ring with LUMO of the other is enhanced. These orbitals would be orthogonal in the mirror image form and the charge-transfer interactions forbidden.

Experimental Section

Nuclear magnetic resonance spectra (Figure 2) were measured on a Varian 220 spectrometer on ~5% solutions using tetramethylsilane as the internal standard. Ultraviolet spectra (Figure 3) at 25° were recorded on a Cary 14 spectrometer. Measurements at other temperatures were carried out using a Cary 17 spectrophotometer with the temperature maintained to ±0.1°. All solvents employed for spectral measurements were of spectro quality.

N,N-**Dimethyl-***N,N'*-**diphenylurea (Ia).**—The compounds *N,N'*-diphenylurea (1.0 g, 4.71 mmol), silver oxide (4.0 g, 7.25 mmol), methyl iodide (4 ml, 64.22 mmol), and *N,N*-dimethyl-

(6) H. H. Jaffe and M. Orchin, "Theory and Applications of Ultraviolet Spectroscopy," Wiley, New York, N. Y., 1962, p 276.
(7) D. J. Cram and H. Steinberg, *J. Amer. Chem. Soc.*, **73**, 5691 (1951).

formamide (25 ml) were stirred at room temperature in a pressure bottle for 24 hr.

After filtering and washing with a few milliliters of *N,N*-dimethylformamide, chloroform (80 ml) was added to the filtrate. After filtration, the filtrate was washed three times with an aqueous solution of 5% potassium cyanide and six times with water and dried on anhydrous magnesium sulfate. Chloroform and *N,N*-dimethylformamide were evaporated; crystalline *N,N'*-dimethyl-*N,N'*-diphenylurea was obtained in 70% yield. The melting point after recrystallization from chloroform was 122° (lit. mp 122°).

***N,N'*-Di(*p*-nitrophenyl)urea (A).**—Two routes were followed for the preparation of A; the first one gave the best yields. The melting points recorded for A show some discrepancies, perhaps owing to the different crystalline forms in which it crystallizes from pyridine and *N,N*-dimethylformamide.[8] Elementary analysis was recorded on a sample crystallized from *N,N*-dimethylformamide.

A.—The compounds *p*-nitroaniline and urea, in the ratio 2:1, were allowed to react in acetic acid and water (1:1) at 130°. The starting amine dissolved slowly; after 18 hr a yellow solid formed, with ammonia evolution. After 24 hr, the solid was filtered and washed with water. The yield varied between 70 and 80%. A was soluble in *N,N*-dimethylformamide, dimethyl sulfoxide, and pyridine, and slightly soluble in acetone (0.5 g of A dissolved in 500 ml of acetone). Crystallization from *N,N*-dimethylformamide gave long, bright, yellow needles, that sublime above 325°.

B.—The compounds *p*-nitroaniline (2.8 g, 20.0 mmol) and *p*-nitrophenyl isocyanate (2.5 g, 15.0 mmol) in benzene (150 ml) and 3–4 drops of triethylamine were refluxed for 5 hr. The precipitated, yellow urea was filtered, dried, and dissolved in pyridine. Such a dissolution was very slow and was possible only after heating. From the first crystallization, 2.5 g (57%) of silky, thin needles were obtained, mp 323° dec.

Anal. Calcd for $C_{13}H_{10}N_4O_5$: C, 51.66; H, 3.34; N, 18.54. Found: C, 51.61; H, 3.24; N, 18.73.

***N,N'*-Dimethyl-*N,N'*-di(*p*-nitrophenyl)urea (Ic).**—The compounds *N,N'*-di(*p*-nitrophenyl)urea (1.0 g, 3.31 mmol), silver oxide (4.0 g, 17.25 mmol), methyl iodide (6 ml, 96.33 mmol), and *N,N*-dimethylformamide (20 ml) were stirred for 20 hr in a pressure bottle at 45°. After the procedure described for I, a crystalline, yellow solid was obtained, mp 145°, yield 80%. After crystallization from acetone, the melting point was 155°.

Anal. Calcd for $C_{15}H_{14}N_4O_5$: C, 54.54; H, 4.24; N, 16.97. Found: C, 54.32; H, 4.09; N, 16.79.

***N*-*p*-Nitrophenyl-*N'*-*p*-methoxyphenylurea.**—The compound *p*-nitrophenyl isocyanate (5.0 g, 30.5 mmol) was placed in a Soxhlet apparatus and extracted with carbon tetrachloride (500 ml) containing *p*-anisidine (3.7 g, 30 mmol). Reflux was maintained for about 1 hr. The precipitated urea was filtered and washed with boiling methanol, yield 7.4 g (86%). Crystallization in dimethyl sulfoxide–water, followed by crystallization in acetone–water, gave mp 229°.

Anal. Calcd for $C_{14}H_{13}N_3O_4$: C, 58.54; H, 4.53; N, 14.65; OCH_3, 10.80. Found: C, 57.95; H, 4.71; N, 14.39; OCH_3, 10.98.

***N,N'*-Dimethyl-*N*-*p*-nitrophenyl-*N'*-*p*-methoxyphenylurea (Id).**—The compounds *N*-*p*-nitrophenyl-*N'*-*p*-methoxyphenylurea (1.0 g, 3.48 mmol), silver oxide (4.0 g, 17.2 mmol), and methyl iodide (9 ml, 144 mmol) in *N,N*-dimethylformamide (25 ml) were stirred for 24 hr at room temperature in a pressure bottle. After the procedure described for I, a viscous oil was obtained, yield 60%. Crystallization in ethyl ether at −20° gave prismatic, light yellow crystals, mp 88°; nmr spectra showed all the resonances in the correct ratio.

***N,N'*-Di(*p*-methoxyphenyl)urea.**—The compounds *p*-methoxyphenyl isocyanate (1.86 g, 13.4 mmol) and *p*-anisidine (2.0 g, 16.2 mmol) in carbon tetrachloride (500 ml) were refluxed for 4–5 hr. The precipitated urea was crystallized from *N,N*-dimethylformamide. After two crystallizations, the yield was 38%, mp 242°.

Anal. Calcd for $C_{15}H_{16}N_2O_3$: C, 66.18; H, 5.88; N, 10.45. Found: C, 65.90; H, 5.99; N, 10.30.

***N,N'*-Dimethyl-*N,N'*-di(*p*-methoxyphenyl)urea (Ib).**—The compound *N,N'*-di(*p*-methoxyphenyl)urea (1.0 g, 3.67 mmol) was methylated following the procedure described for I and II. The melting point of the crude, methylated urea was 79°. The urea is soluble in chloroform and acetone, but in these solvents, even at −20°, no crystallization occurred. The compound was crystallized from *n*-hexane, and the melting point after two crystallizations was 86°. The yield after the first crystallization was 80%. Examination of the product using nmr showed all the expected resonances in the correct ratio.

(8) S. A. Buckler, *J. Org. Chem.*, **24**, 1460 (1959).

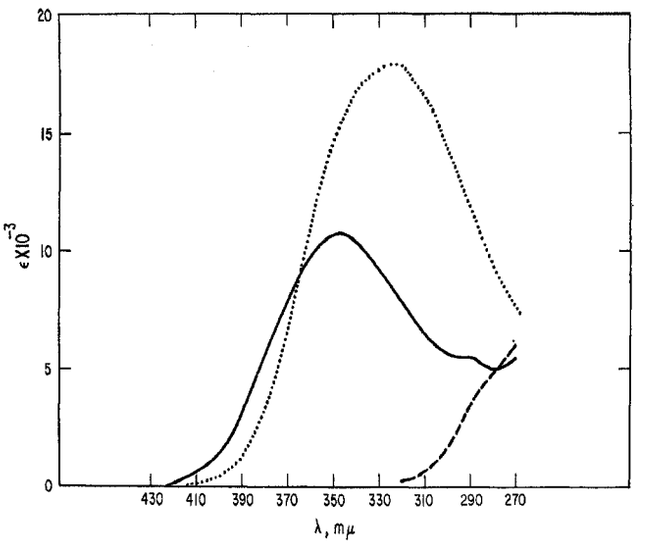


Figure 3.—Ultraviolet spectra for *N,N'*-dimethyl-*N,N'*-di(*p*-nitrophenyl)urea (dotted line), *N,N'*-dimethyl-*N*-*p*-nitrophenyl-*N'*-*p*-methoxyphenylurea (solid line), and *N,N'*-dimethyl-*N,N'*-di(*p*-methoxyphenyl)urea (dashed line), at 25° in chloroform.

***N,N*-Dimethyl-*N'*-methyl-*N'*-phenylurea (IIa).**—The compounds phenylurea (1.0 g, 7.35 mmol), silver oxide (6.0 g, 25.8 mmol), methyl iodide (7 ml, 112 mmol), and *N,N*-dimethylformamide (25 ml) were stirred at room temperature for 3 days in a pressure bottle.

After filtering, chloroform was added to the filtrate until no precipitation of silver iodide occurred. The solvent was eliminated under vacuum and the oil obtained ws purified on preparative layer chromatography plates of silica gel with a mixture of hexane–ethyl acetate (3:2).

Two main products were observed; that one with the longer R_f was recognized as IIa by nmr integration. The other one with a longer R_f was found to be *N*-*N'*-dimethyl-*N*-phenylurea, again by nmr integration.

***N,N*-Diethyl-*N'*-ethyl-*N'*-nitrophenylurea (IIc).**—The compounds *N*-ethyl-*N*-*p*-nitrophenylcarbamoyl chloride (0.5 g, 2.2 mmol), diethylamine (20 ml), and diglime (14 ml) were refluxed for 18 hr. Diethylamine hydrochloride was filtered off, and the filtrate was evaporated; the product was obtained in 87% yield. After crystallization from ethyl bromide, the melting point was 50°.

Anal. Calcd for $C_{12}H_{16}N_3O_3$: C, 58.87; H, 7.17; N, 15.84. Found: C, 58.9; H, 7.32; N, 15.63.

***N,N*-Diethyl-*N'*-ethyl-*N'*-*p*-methoxyphenylurea (IIb).**—The compounds *N*-ethyl-*N*-methoxycarbamoyl chloride (0.5 g, 2.36 mmol), diethylamine (20 ml), and diglime (15 ml) were refluxed for 18 hr. After diethylamine hydrochloride was filtered off, the filtrate was evaporated, and the residue was taken into hexane and cooled to −60° overnight. Crystals were obtained in 66% yield. Recrystallization from ethyl bromide by slow evaporation gave crystals with mp 43°.

Anal. Calcd for $C_{14}H_{22}N_2O_2$: C, 67.20; N, 8.80; H, 11.20. Found: C, 67.01; N, 8.68; H, 10.80.

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Registry No.—A, 587-90-6; Ia, 611-92-7; Ib, 27281-95-4; Ic, 34594-47-3; Id, 40387-31-3; IIa, 32773-27-6; IIb, 40387-32-4; IIc, 40387-33-5; *N,N'*-diphenylurea,

102-07-8; methyl iodide, 74-88-4; *p*-nitroaniline, 100-01-6; urea, 57-13-6; *p*-nitrophenyl isocyanate, 100-28-7; *N*-*p*-nitrophenyl-*N'*-*p*-methoxyphenylurea, 40387-34-6; *p*-anisidine, 104-94-9; *N*,*N'*-di(*p*-methoxyphenyl)-urea, 1227-44-7; *p*-methoxyphenyl isocyanate, 5416-93-3; phenylurea, 64-10-8; *N*-ethyl-*N*-*p*-nitrophenylcarbamoyl chloride, 34208-12-3; diethylamine, 109-89-7; *N*-ethyl-*N*-methoxycarbamoyl chloride, 40387-35-7.

Protection of Tryptophan with the Formyl Group in Peptide Synthesis[1]

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N^{α}-*tert*-Butyloxycarbonyl-N^{i}-formyltryptophan has been synthesized and used for the solid-phase synthesis of the heptapeptide Gly-Ala-Arg-Gly-Ala-(formyl)Trp-Gly which was isolated in high yield. Removal of the formyl group in 0.01 *M* ammonium bicarbonate buffer of pH 9 was accompanied by an unexpected side reaction, but this could be greatly diminished by use of 1 *M* buffer. The overall yield of the deprotected heptapeptide was substantially higher than in a parallel synthesis where tryptophan was not protected. The formyl group can also be effectively removed with little side reaction in liquid ammonia containing hydroxylamine hydrochloride.

Synthesis of tryptophan-containing peptides has been handicapped by lack of a protecting group for the indole side chain. Destruction of tryptophan in synthesis has generally been regarded to occur during the acid treatments for removal of protecting groups. Butylation of the indole moiety during this step constitutes a serious danger.[2] Use of HCl–acetic acid together with mercaptoethanol as scavenger[3] have been recommended as deprotecting agent for removal of the N^{α}-Boc group,[4] but it has been reported recently that these tactics are ineffective in solid-phase peptide synthesis and lead to a heterogeneous product.[5] On the other hand, use of HCl–formic acid as deprotecting agent gave a nearly homogeneous peptide.[5] Since reversible modification of tryptophan with the formyl group has already been described,[6] the conclusion was reached that this protection might be suitable in peptide synthesis. We wished to explore this possibility under our synthetic conditions as part of efforts to develop a complete set of side-chain protecting groups for use with N^{α}-Boc protection in solid-phase synthesis of peptides.[7] We have now synthesized the model heptapeptide Gly-Ala-Arg-Gly-Ala-Trp-Gly (I) with and without formyl protection of tryptophan and have found the protection to be well suited for peptide synthesis. Removal of the formyl group led to unexpected side reaction but conditions were established to reduce this to a minimum.

One of the most reliable means for removal of the N^{α}-Boc group in solid-phase synthesis has been 50% trifluoroacetic acid in dichloromethane.[8,9] The reagent was not recommended for tryptophan-containing peptides.[3] We decided to synthesize peptide I with use of this reagent to ascertain the extent of the problem. Boc-Glycyl resin was prepared by a modified Loffet procedure.[10,11] N^{α}-Boc protection was used along with N^{G}-tosyl protection of arginine. Removal[12] of protecting groups and the solid support in HF gave a product which proved to be heterogeneous on gel filtration on Sephadex G-10 (Figure 1a). The fast-moving side-product (Figure 1a) was purified in carboxymethylcellulose[13] and gave an ultraviolet spectrum similar to that reported for peptides containing an altered tryptophan residue.[5] Peptide I required further purification on carboxymethylcellulose and by partition chromatography[14] on Sephadex G-25 before its isolation in highly purified form[15] (yield *ca.* 23% based on starting Boc-glycyl resin.).

It has been shown that N^{i}-formyltryptophan or a suitable derivative is stable in solution under the acidic and basic conditions used for solid-phase synthesis including treatment with HF.[5] We therefore decided to attempt synthesis and isolation of the formyl derivative of I, namely Gly-Ala-Arg-Gly-Ala-(formyl)Trp-Gly (II). For this purpose N^{α}-Boc-N^{i}-formyltryptophan was required. This derivative was prepared from N^{i}-formyltryptophan[5] by the dimethyl sulfoxide procedure[16] and isolated as its crystalline dicyclohexylamine salt. Its ultraviolet spectrum was in agreement with that expected for a derivative of N^{i}-formyltryptophan. Synthesis of II was carried out by procedures entirely analogous to those used for synthesis of I, including treatment of the finished peptide resin with HF. Gel filtration of the product on Sephadex G-10 gave a single peak (Figure 1b). Further chromatography on carboxymethylcellulose in which only a single peak was detected gave peptide II in highly purified

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