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o-Chloranil–Azlactone Adducts and Their Conversions to Unsaturated Amino Acid Derivatives

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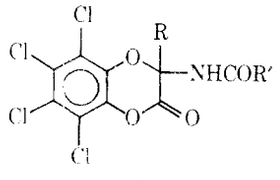
The synthesis of a series of tetrachlorodioxinones (1) and their conversions into α-substituted and dehydro amino acid derivatives is discussed. The first synthesis of a dehydro dipeptide derivative from a dipeptide is also reported.

The synthesis of dehydro amino acids and peptides has been of interest to us[2] and to others[3] for some time. In a recent preliminary report,[2c] we described the synthesis of some chlorodioxinones by the reaction of amino acid azlactones with *o*-chloranil. This paper describes that work in more detail and the conversion of the dioxinones into unsaturated amino acid derivatives.

When an acetic anhydride solution of an *N*-benzoyl amino acid was treated with an equimolar amount of *o*-chloranil at room temperature, the dioxinones (**1**, Table I) crystallized from the solution in 60–95% yields. If the azlactone was formed by treatment of the *N*-acyl amino acid with *N,N'*-dicyclohexylcarbodiimide in an inert solvent, the adducts (**1**) were



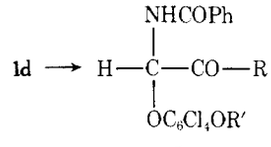
1

a, R = $PhCH_2$; R' = Ph
b, R = $(CH_3)_2CH$; R' = Ph
c, R = CH_3; R' = Ph
d, R = H; R' = Ph
e, R = Ph; R' = Ph
f, R = $(CH_3)_2CHCH_2$; R' = Ph
g, R = $PhCH_2$; R' = CH_3

formed in somewhat lower yields than when acetic anhydride was used. The off-resonance ^{13}C NMR spectra of **1a** and **1d** showed an 88.6-ppm singlet and a 75.2-ppm doublet for C-3 of **1a** and **1d**, respectively. These data along with infrared and ^1H NMR spectra confirmed the dioxinone structure of these compounds.

The chemistry of these compounds was also consistent with the dioxinone structure. They were rapidly converted into α-substituted amino acid derivatives when treated with nucleophiles such as CH_3O^-, $PhNH_2$, and $PhCH_2SH$ and these products are shown in Tables II and III.

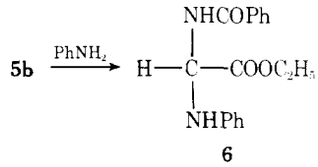
The chemistry of **1d**, formed from *N*-benzoylglycine, was considerably different from that of **1** derived from the other amino acids having an R group larger than hydrogen. The protio compound (**1d**) reacted with water, ethanol, and aniline very rapidly at room temperature giving the α-chlorophenoxy acid salt (**5a**), ester (**5b**), and anilide (**5c**) in excellent yields.



5

a, R = $O^-Et_3\overset{+}{N}H$; R' = H
b, R = OEt; R' = H
c, R = NHPh; R' = H
d, R = OEt; R' = CH_3

Since the α-chlorophenoxy group is an excellent leaving group, compounds of the type **5** could be converted into other α-substituted amino acid derivatives as shown below. In this way



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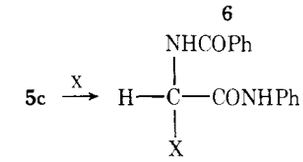
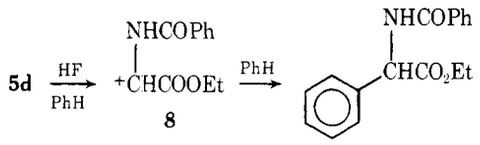


7a, X = CH_3O
b, X = PhNH
c, X = $PhCH_2S$

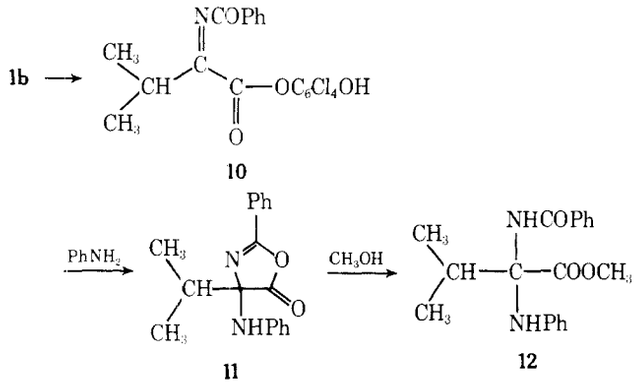
compounds having *different* groups attached to the α- and carbonyl carbon atoms could be prepared. The ether (**5d**), formed by treatment of **5b** with diazomethane, reacted with anhydrous hydrogen fluoride in benzene solution to give an excellent yield of the known phenylglycine derivative **9**. This



8 9

reaction undoubtedly proceeds through an α-carbonium ion (8) as postulated by Ben-Ishai for α-hydroxy-[4a] and α-alkoxyglycine[4b] reactions with nucleophiles under acid conditions.

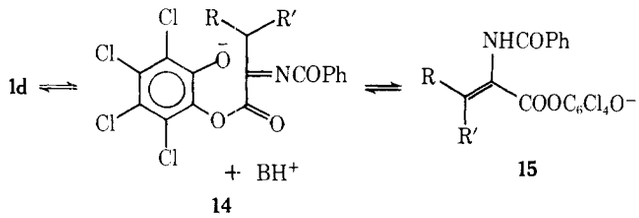
The dioxinones **1a** (R = $PhCH_2$) and **1b** [R = $(CH_3)_2CH$] did not react with alcohols in the absence of alkoxides, while **1d** (R = H) reacted rapidly. Since **1a** and **1b** have sterically hindered carbonyl groups (trisubstituted α-carbon atom), reaction at this site was very slow. Under basic conditions, however, both **1a** and **1b** reacted rapidly to give α-alkoxy esters. We observed that when **1b** was treated with 1 equiv of aniline, an intermediate having a high-frequency carbonyl absorption at 1825 cm^{-1} was formed. The addition of methanol to this solution converted the intermediate into the α-anilino methyl ester (**12**). These facts are consistent with the formation of an acylimine intermediate (**10**) as a first step. Cyclization to the azlactone **11** by attack of aniline at the azomethine carbon of **10** accounts for the observed high frequency carbonyl absorption. Methanolysis of **11** then finally gives **12**. It was generally found that the dioxinones gave



10 11 12

complex mixtures with a single molar equivalent of amines and that at least 1 more equiv was necessary in order to obtain good yields of α-amino amides (**3**). The liberation of the phenolic hydroxyl group in **10** probably neutralizes part of the amine slowing the reaction, and the liberation of tetrachlorocatechol in the formation of **11** requires at least 1 mol of amine for neutralization.

Since we were interested in the synthesis of dehydro amino acid derivatives, we hoped to convert the quinone adducts into such compounds. The possible equilibrium formation of acylimines (**14**) from **1** using nonnucleophilic bases should[3c]



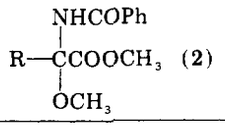
14 15

allow their isomerization to the desired α,β-unsaturated compounds (**15**). We felt that O-methylation of **14** would prevent its return to **1** and allow even a slow equilibration to an *O*-methyl-**15** to occur. Consequently, several adducts were treated with methyl iodide/anhydrous potassium carbonate in acetone at room temperature. Instead of a methyl ether of **14** or **15**, the unsaturated acylamino[5] esters (**16a–c**) were isolated in excellent yields along with tetrachlorocatechol dimethyl ether. The formation of these products from **1** clearly requires the presence of water or methanol in the reaction mixture, since water would convert **15** into the unsaturated acid which would be methylated (esterified) and methanol would afford the ester **16** directly. We took precautions to remove all water from the reaction mixture and, in the light of our experience in the hydrolysis of **1**, we felt that excess water would rapidly convert the intermediate acylimine **14** into an unstable α-hydroxy compound which would decompose spontaneously into the amide and α-keto derivative. In our opinion, the excellent yields of **16** precluded the presence of water. Using NMR spectroscopy, we found that methyl iodide did not react with potassium carbonate in acetone-d_6 over a period of 1 week, but when acetic acid was added both methyl acetate and water were formed in a matter of hours. Apparently, a proton source such as acetic acid or **1** converted potassium carbonate into carbon dioxide and water rather than to potassium bicarbonate as we had expected. The rate

Table I

Compd	Yield, %	Mp, °C	Solvent
1a	73	237–238	Me_2SO
1b	90	200–201	EtOAc–hexane (1:4)
1c	95	228–229	EtOAc–hexane
1d	61	229–230	$CHCl_3$
1e	93	201–203	EtOAc–petroleum ether (1:1)
1f	88	220–221	EtOAc–hexane

Table II

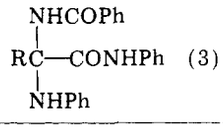


(2)

Compd	Yield, %	Mp, °C	Solvent
2a	77	125–126	EtOH–H_2O(4.5:1)
2b	70	74–75	Et_2O–petroleum ether (3:1)
2d	80	78–80[a]	Et_2O
2e	83	118–119	Et_2O

[a] Reference 7, mp 87–88 °C.

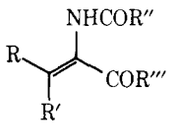
Table III



(3)

Compd	Yield, %	Mp, °C	Solvent
3a	61	185–187[a]	CH_3OH–H_2O
3b	69	168–169	CH_3OH–H_2O
3d	61	167–168[b]	CH_3OH

[a] **3a** melted at 171–173 °C, resolidified, and remelted as shown. [b] Reference 7, mp 163–164 °C.

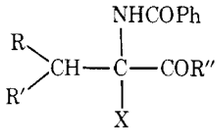


16

a, R = Ph; R′ = H; R″ = CH_3; R‴ = OCH_3
b, R = R′ = CH_3; R″ = Ph; R‴ = OCH_3
c, R = $(CH_3)_2CH$; R′ = H; R″ = Ph; R‴ = OCH_3
d, R = Ph; R′ = H; R″ = Ph; R‴ = OCH_3
e, R = R′ = CH_3; R″ = Ph; R‴ = NHPh

of isomerization, **14** → **15**, was apparently so fast that only **15** was available to react with the water present.

In further work, we found that the α-substituted amino acid derivatives (**17a–c**) underwent elimination in hot pyridine

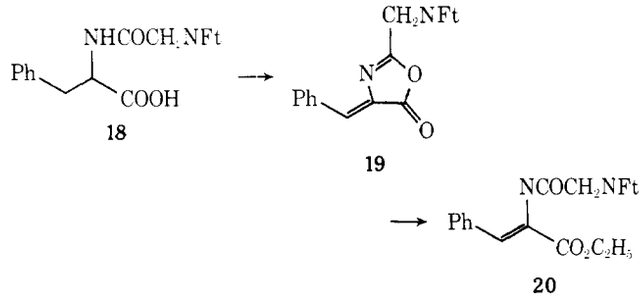


17

a, R = Ph; R′ = H; R″ = OCH_3; X = OCH_3
b, R = R′ = CH_3; R″ = OCH_3; X = OCH_3
c, R = R′ = CH_3; R″ = NHPh; X = NHPh

giving the unsaturated amino acid derivatives (**16b,d,e**). A recent report[3k] by Schmidt describes a similar elimination catalyzed by methoxide ion. Thus, **1** can be converted directly into a dehydro amino acid derivative or through an α-substituted intermediate like **17**.

More interestingly, we were able to prepare an *N*-acyl unsaturated dipeptide directly from the corresponding N-protected saturated compound. When *N*-phthaloylglycyl-DL-phenylalanine (**18**) was treated with *o*-chloranil in acetic anhydride solution, the unsaturated azlactone (**19**) was obtained in 50% yield after recrystallization of the crude precipitate. None of the expected dioxinone adduct was isolated from this reaction. Conversion of **19** into the known[7] ester **20** by ethanolysis confirmed the structure of **19** as drawn. We believe that this is the first report of a direct oxidation of a dipeptide derivative to form a dehydropeptide. We are presently studying the extension of this oxidation procedure to other peptides of biological interest.



18 **19** **20**

Ft = phthaloyl

Experimental Section

General. All melting points were determined on a Nalge Model Y6 micro hot stage and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer Model 257 recording spectrometer with polystyrene as the standard. The ^1H NMR spectra were recorded on a Perkin-Elmer T-60 spectrometer or on a Varian 90-MHz spectrometer with tetramethylsilane as internal standard, and the ^{13}C NMR spectra were determined on a JOEL-PFT-100 spectrometer with Me_4Si as the internal standard; chemical shifts were obtained by computer output. The ultraviolet–visible spectra were determined on a Perkin-Elmer Model 202 spectrophotometer. Elemental analyses were carried out by Atlantic Microlabs, Atlanta, Ga.

General Procedure for the Synthesis of 3-Acylamino 3-Substituted 5,6,7,8-Tetrachloro-1,4-benzodioxin-2(3*H*)-ones (1). A mixture of 20 mmol of *N*-acylamino acid in 20–25 ml of acetic anhydride was warmed until homogeneous and 20 mmol of *o*-chloranil was added. The solution was allowed to stand overnight, cooled in an ice bath, and filtered to give 60–100% yields of the crude crystalline dioxinones (**1**). Recrystallization solvents are given in Table I. The dioxinones all showed the following major IR bands (Nujol): 3230–3370 (N–H), 1765–1805 (C=O), 1655–1670 cm^{-1} (CONH).

Spectra (Off-Resonance) in Me_2SO-d_6. ^{13}C NMR. 1a: 169.9 (s, C=O), 162.0 (s, C=O), 140.8–120.6 (m, C_6H_5), 88.6 (s, CNHCOPh), 45.1 (t, CH_2Ph). **1d:** 167.3 (s, C=O), 159.6 (s, C=O), 143.4–127.7 (m, C_6H_5), 75.2 (d, CHNHCOPh).

3-Acetamido-3-benzyl-5,6,7,8-tetrachloro-1,4-benzodioxin-2(3*H*)-one (1g). A mixture of 8.42 g (34.3 mmol) of *o*-chloranil and 6.20 g (37.5 mmol) of DL-phenylalanine in 20 ml of acetic anhydride was stirred overnight at room temperature and then cooled at −5 °C for 6 h and filtered, and the solid was dried in vacuo giving 7.35 g (49%) of crude **1a**, mp 224–226 °C dec. Crystallization from ethyl acetate–hexane gave an analytical sample: mp 226–228 °C dec; IR (Nujol) 3250 (NH), 3200 (NH), 1790 (C=O), 1650 (amide C=O), 1540 cm^{-1} (amide II); NMR (Me_2SO-d_6) δ 10.12 (s, 1 H, NH), 7.19 (s, 5 H, C_6H_5), 3.49 (s, 2 H, CH_2), 2.00 ppm (s, 3 H, CH_3).

Anal. Calcd for $C_{17}H_{11}NCl_4O_4$: C, 46.93; H, 2.55; N, 3.22. Found: C, 46.99; H, 2.55; N, 3.31.

General Procedure for Synthesis of *N*-Acyl-α-methoxy Amino Acid Methyl Esters (2). To a methanol suspension of the dioxinone (2–6 mmol) was added 5 ml of 0.5 N $KOCH_3/CH_3OH$ solution and the mixture was stirred magnetically at room temperature until homogeneous (1–16 h, 5 days for **1a**). The solution was evaporated in vacuo and the residue was extracted with methylene chloride. The extracts were washed twice with 5% sodium carbonate solution containing sodium dithionite, dried (anhydrous Na_2SO_4), and evaporated in vacuo to give the crude **2** which was recrystallized. See Table II. Compound **1d** was chromatographed on a silica gel (60–200 mesh) column by elution with $CHCl_3$. The products (**2**) all showed IR (Nujol) bands at 3270–3320 (NH), 1745 (C=O) or 1720–1740, 1765–1770 paired peaks (C=O, **2a** and **2d**), 1650–1675 cm^{-1} (CONH).

General Procedure for Synthesis of *N*-Acyl-α-anilino Amino Acid Amides (3). One millimole of dioxinone was treated with 2–7 mmol of aniline in 50–100 ml of CH_2Cl_2 at room temperature for 16 h. The solution was washed with 2 × 50 ml of 5% sodium carbonate solution containing sodium dithionite, dried (anhydrous Na_2SO_4), and evaporated in vacuo giving the crude crystalline **3** which was recrystallized (Table III). The IR spectra (Nujol) of these products (**3**) showed three bands in the 3290–3440-cm^{-1} region (N–H) and two bands near 1660 and 1695 cm^{-1} (CONH).

Benzyl *N*-Benzoyl-α-benzylmercaptothiolglycinate (4). A solution of 407 mg (1 mmol) of **1d** and 296 mg (2.39 mmol) of benzyl mercaptan in 75 ml of methylene chloride containing 5 drops of triethylamine was stirred for 2 days at room temperature. The reaction mixture was concentrated in vacuo and the crude oil was purified on a column (4.2 × 50 cm) of silica gel (60–200 mesh) by elution with chloroform giving 342 mg (84%) of **4**, mp 106–107 °C. Crystallization from methanol gave an analytical sample: mp 106–107 °C (lit.[7] mp 103–104 °C); IR (Nujol) 3340 (NH), 1690 cm^{-1} (C=O); NMR (Me_2SO-d_6) δ 9.49 (d, 1 H, CHN**H**, J = 8 Hz), 7.68 (m, 5 H, C_6H_5CONH–), 7.26 (s, 10 H, 2 $C_6H_5CH_2S$), 5.83 (d, 1 H, C**H**NH, J = 8 Hz), 4.12 (s, 2 H, SCH_2Ph), 3.94 ppm (s, 2 H, SCH_2Ph).

Triethylammonium *N*-Benzoyl-α-(2-hydroxy-3,4,5,6-tetrachlorophenoxy)glycinate (5a). A solution of 952 mg (2.34 mmol) of **1d** and 20 ml of THF containing 2.34 mmol of H_2O and 2.34 mmol of triethylamine was allowed to stand at room temperature for 6 h. The solvent was evaporated in vacuo, giving 1.25 g of an amorphous solid. Crystallization of the crude solid from ethyl acetate–hexane gave 905 mg (91%) of an analytical sample of **5a**: mp 115–126 °C; IR (Nujol) 3240 and 3190 (NH), 1680 (amide C=O), 1600 cm^{-1} (COO^-); NMR ($CDCl_3$) δ 8.35 (d, 1 H, CHN**H**, J = 8 Hz), 7.81 (m, 2 H, ortho H's of C_6H_5), 7.37 (m, 3 H, meta and para H's of C_6H_5), 5.88 (d, 1 H, NHC**H**, J = 8 Hz), 3.09 (q, 6 H, J = 7 Hz, CH_2CH_3), 1.26 ppm (t, 9 H, J = 7 Hz, CH_2CH_3).

Anal. Calcd for $C_{21}H_{24}N_2O_5Cl_4$: C, 47.93; H, 4.60; N, 5.32. Found: C, 47.80; H, 4.67; N, 5.42.

***N*-Benzoyl-α-(2-hydroxy-3,4,5,6-tetrachlorophenoxy)glycine Ethyl Ester (5b).** A solution of 1.76 g (4.3 mmol) of **1d** in 200 ml of methylene chloride containing 14 ml of ethanol and several drops of acetic acid was stirred at room temperature for 2 h. The reaction was complete when the C=O absorption at 1805 cm^{-1} disappeared. The reaction mixture was evaporated to dryness in vacuo, giving 1.86 g (95%) of crude **5b**, mp 136–142 °C. Crystallization from ethyl acetate–hexane gave an analytical sample: mp 149.5–151 °C dec; IR (Nujol) 3420 (OH), 3320 (NH), 1750 (C=O), 1645 cm^{-1} (amide C=O); NMR (Me_2SO-d_6) δ 9.72 (d, 1 H, CHN**H**, J = 9 Hz), 7.66 (m, 5 H, C_6H_5), 6.37 (d, 1 H, C**H**NH, J = 9 Hz), 4.30 (q, 2 H, CH_2CH_3), 1.27 ppm (t, 3 H, CH_2CH_3).

Anal. Calcd for $C_{17}H_{13}NO_5Cl_4$: C, 45.04; H, 2.89; N, 3.09. Found: C, 45.03; H, 2.92; N, 3.16.

***N*-Benzoyl-α-(2-hydroxy-3,4,5,6-tetrachlorophenoxy)glycine Anilide (5c).** A solution of 507 mg (1.25 mmol) of **1d** and 116 mg (1.25 mmol) of aniline in 125 ml of methylene chloride was stirred at room temperature for 30 min. The reaction was complete when the C=O absorption at 1805 cm^{-1} had disappeared. After the solution was cooled to −5 °C overnight, the precipitate was filtered and dried in vacuo, giving 564 mg (90%) of **5c**, mp 171–172 °C dec. Recrystalliza-

tion from methylene chloride gave an analytical sample: mp 171–172 °C dec; IR (Nujol) 3400 (OH), 3320 and 3270 (NH), 1680 (amide C=O), 1640 cm^{-1} (amide C=O); NMR (Me_2SO-d_6) δ 10.27 (s, 1 H, NHPh), 9.69 (d, 1 H, CHN**H**, J = 9 Hz), 7.51 (m, 10 H, 2 C_6H_5), 6.51 ppm (d, 1 H, C**H**NH, J = 9 Hz).

Anal: Calcd for $C_{21}H_{14}N_2O_4Cl_4$: C, 50.43; H, 2.82; N, 5.60. Found: C, 50.33; H, 2.82; N, 5.69.

Ethyl 2-(2,3,4,5-Tetrachloro-5-methoxyphenoxy)-2-benzamidoacetate (5d). A diazomethane (ca. 0.3 g)–ether solution was added to a solution of **5b** (2.27 g) in 20 ml of CH_2Cl_2 at room temperature. After 10 min, the mixture was evaporated in vacuo to give an oil which was crystallized by addition of petroleum ether. The crude product was recrystallized from ether–hexane to afford 1.90 g (81%) of colorless needles: mp 125–127 °C; IR (Nujol) 3200 (NH), 1745 (ester), 1640 cm^{-1} (amide); NMR ($CDCl_3$) δ 7.2–7.8 (m, 6 H, C_6H_5 and NH), 6.64 (d, 1 H, J = 10, CHN), 4.36 (q, 2 H, OCH_2), 3.95 (s, 3 H, OCH_3), 1.35 ppm (t, 3 H, OCH_2CH_3).

Anal. Calcd for $C_{18}H_{15}NO_5Cl_4$: C, 46.28; H, 3.24; N, 3.00. Found: C, 46.10; H, 3.24; N, 3.04.

***N*-Benzoyl-α-anilinoglycine Ethyl Ester (6).** A mixture of 592 mg (1.46 mmol) of **1d,** 6 ml of absolute ethanol, and 2 drops of acetic acid in 100 ml of methylene chloride was stirred at room temperature for 3 h. When the C=O absorption at 1805 cm^{-1} had disappeared, 0.2 ml (2 mmol) of aniline was added. After stirring for 3 days at room temperature, the reaction mixture was washed with two 50-ml portions of 5% sodium carbonate containing sodium dithionite and dried (Na_2SO_4). The solvent was evaporated in vacuo, giving 454 mg of crude yellow **6.** Crystallization from 2:1 ethanol–H_2O gave 283 mg (70%) of **6,** an analytical sample: mp 130–131 °C; IR (Nujol) 3400, 3315 (NH), 1740 (C=O), 1640 (amide C=O), 1610 cm^{-1} (NH); NMR (Me_2SO-d_6) δ 9.20 (d, 1 H, J = 8 Hz, CHN**H**), 7.84–6.92 (m, 10 H, 2 C_6H_5), 6.12 (d, 1 H, J = 8 Hz, C**H**NH), 4.18 (q, 2 H, OCH_2CH_3), 1.19 ppm (t, 3 H, OCH_2CH_3).

Anal. Calcd for $C_{17}H_{18}N_2O_3$: C, 68.44; H, 6.08; N, 9.39. Found: C, 68.59; H, 6.10; N, 9.32.

***N*-Benzoyl-α-methoxyglycine Anilide (7a).** A solution of 867 mg (1.73 mmol) of **5c** in 50 ml of methanol containing 10 ml of 0.5 N potassium methoxide was allowed to stand at room temperature for 1 h. The solvent was evaporated in vacuo, the residue was suspended in 50 ml of methylene chloride, and the mixture was washed with two 50-ml portions of 5% sodium carbonate containing sodium dithionite and was dried (Na_2SO_4). The solvent was evaporated in vacuo, giving 416 mg (86%) of crude **7a,** mp 132.5–134 °C. Crystallization from 1:1 methanol–H_2O gave 400 mg (82%), an analytical sample: mp 134–135 °C (lit.[7] mp 135–136 °C); IR (Nujol) 3330, 3270 (NH), 1695 (amide C=O); NMR (Me_2SO-d_6) δ 9.94 (s, 1 H, PhN**H**CO), 9.16 (d, 1 H, CHN**H**, J = 8 Hz), 7.52 (m, 10 H, 2 C_6H_5), 5.68 (d, 1 H, C**H**NH, J = 8 Hz), 3.43 ppm (s, 3 H, OCH_3); ^{13}C NMR (off-resonance) (Me_2SO-d_6) 167.1 (s, C=O), 165.9 (s, C=O), 138.3–119.8 (m, C_6H_5), 80.2 (d, CH), 55.4 (q, CH_3).

***N*-Benzoyl-α-benzylmercaptoglycine Anilide (7c).** A mixture of 443 mg (0.884 mmol) of **5c** in 50 ml of methylene chloride containing 1.02 mmol of benzyl mercaptan and several drops of triethylamine was stirred at room temperature overnight. The reaction mixture was washed with two 50-ml portions of 5% sodium carbonate containing sodium dithionite and dried (Na_2SO_4), and the solvent was evaporated in vacuo, giving a solid residue. Crystallization from methanol gave 214 mg (64%) of **7c,** mp 197–199.5 °C. Further recrystallization from methanol afforded an analytical sample: mp 200–201 °C; IR (Nujol) 3260 (NH), 1655 (amide C=O), 1635 cm^{-1} (amide C=O); NMR (Me_2SO-d_6) δ 10.38 (s, 1 H, PhN**H**), 8.92 (d, 1 H, CHN**H**, J = 8 Hz), 7.50 (m, 15 H, 3 $C_6\mathbf{H}_5$), 5.96 (d, 1 H, C**H**NH), J = 8 Hz), 4.12 (unsymmetrical doublet, J_{ax} = 18 Hz, SCH_aH_xPh) and 3.98 ppm (unsymmetrical doublet, J_{ax} = 18 Hz, SCH_aH_xPh).

Anal. Calcd for $C_{22}H_{20}N_2O_2S$: C, 70.19; H, 5.36; N, 7.44. Found: C, 70.07; H, 5.45; N, 7.46.

Ethyl *N*-Benzoylphenylglycinate (9) from 5d. A solution of **5d** (234 mg) in 10 ml of dry benzene was saturated with dry HF at room temperature. After 10 min, the mixture was evaporated in a stream of dry N_2, the residue was dissolved in ether, and the solution was washed with 10% Na_2CO_3 solution. Evaporation of the organic phase gave crystals which were recrystallized from hexane to afford 120 mg (85%) of **9:** mp 87.5–88 °C (lit.[8] mp 89 °C); IR (Nujol) 3350 (NH), 1740 (ester), 1640 cm^{-1} (amide). The basic washing was acidified with HCl to give 100 mg (78%) of tetrachlorocatechol monomethyl ether, mp 121–123 °C (lit.[9] mp 123–124 °C). Hydrolysis of **9** in boiling concentrated HCl for 2 h gave DL-phenylglycine hydrochloride, whose IR was identical with that of an authentic sample.

***N*-Benzoyl-α-anilinovaline Methyl Ester (12).** A solution of 1.19 g (2.65 mmol) of **1b** in 13 ml of methylene chloride containing 7.5 mmol of triethylamine and 2.65 mmol of aniline was allowed to stand at room temperature, and the reaction was followed by TLC and IR. When complete (IR absorption showed a C=O at 1825 cm^{-1}), 1 ml of methanol was added to the solution which was allowed to stand at room temperature for 6 h. After the addition of 50 ml of methylene chloride, the solution was washed with three 50-ml portions of 5% sodium carbonate containing sodium dithionite and dried (Na_2SO_4), and the solvent was evaporated in vacuo, giving a mixture of products. Crystallization from methanol–H_2O gave 323 mg (37%) of crude **12,** mp 141–145 °C. Recrystallization from methanol–H_2O gave 238 mg of an analytical sample of **12:** mp 162.5–164.5 °C; IR (Nujol) 3420, 3440, and 3260 (NH), 3310, 3180 sh (NH), 1745 (C=O), 1640 cm^{-1} (amide C=O); NMR ($CDCl_3$) δ 7.70–6.56 (m, 11 H, 2 C_6H_5, NH), 5.13 (broad s, 1 H, NH), 3.83 (s, 3 H, OCH_3), 3.13 [m, 1 H, $(CH_3)_2C\mathbf{H}$], 1.17 (d, 3 H, J = 7 Hz, $\mathbf{H}_3CH$), 0.99 ppm (d, 3 H, J = 7 Hz, $\mathbf{H}_3CH$).

Anal. Calcd for $C_{19}H_{22}N_2O_3$: C, 69.92; H, 6.79; N, 8.58. Found: C, 70.09; H, 6.84; N, 8.62.

Methyl α-Benzamido-β,β-dimethylacrylate (16b). Method A. From 1b. A mixture of 634 mg (1.41 mmol) of **1b,** 500 mg (3.61 mmol) of finely divided anhydrous (dried 150 °C) potassium carbonate, and 0.18 ml (2.9 mmol) of methyl iodide in 8 ml of acetone was stirred at room temperature for 2 days. The reaction mixture was filtered, and the solvent was evaporated in vacuo. The residue was purified by chromatography on a column (4.2 × 75 cm) of silica gel (60–200 mesh) by elution with 1:1 chloroform–hexane giving 331 mg (96%) of tetrachlorocatechol dimethyl ether, mp 92–93 °C, followed by 291 mg (100%) of **16b,** mp 129–130.5 °C. Crystallization from benzene gave 235 mg: mp 129–130.5 °C, resolidified and remelted at 137–138 °C (lit.[10] mp 137–138 °C); IR (Nujol) 3260 (NH), 1720 (C=O), 1640 cm^{-1} (amide C=O); NMR ($CDCl_3$) δ 8.13–7.85 (m, 3 H, NHCOPh, ortho H's of C_6H_5), 7.65–7.33 (m, 3 H, meta and para H's of C_6H_5), 3.74 (s, 3 H, OCH_3), 2.18 (s, 3 H, CH_3C=), and 1.88 ppm (s, 3 H, CH_3C=).

Method B. From *N*-Benzoyl-α-methoxyvaline Methyl Ester (17b). A solution of 303 mg (1.14 mmol) of **17b** in 2 ml of pyridine was refluxed for 2 h. The solvent was evaporated in vacuo, and the crude solid was crystallized from benzene, giving 256 mg (96%) of **16b,** mp 136–137 °C, spectrally identical with a sample obtained by method A.

Methyl α-Benzamidocinnamate (16d). A solution of 307 mg (0.98 mmol) of **17a** in 2 ml of pyridine was refluxed for 3 h. The solvent was concentrated in vacuo, and the crude oil residue was purified on a column (4.2 × 30 cm) of silica gel (60–200 mesh). Elution with chloroform gave 180 mg (65%) of crude **16d** which was crystallized from ethanol–H_2O giving 100 mg: mp 137–139 °C (lit.[11] mp 140–141 °C); IR (Nujol) 3330 (NH), 1715 (C=O), 1655 cm^{-1} (amide C=O).

Methyl (*Z*)-α-Benzamido-β-isopropylacrylate (16c). A mixture of 1.19 g (2.57 mmol) of **1f,** 1.5 g (10.8 mmol) of finely divided anhydrous potassium carbonate, and 0.75 ml (12 mmol) of methyl iodide in 6 ml of acetone was stirred at room temperature for 2 days. The reaction mixture was filtered, and the solvent was evaporated in vacuo. The residue was separated on a column (4.2 × 75 cm) of silica gel (60–200 mesh) by elution with 1:1 chloroform–hexane, giving 601 mg (85%) of tetrachlorocatechol dimethyl ether, mp 92–93 °C, and 541 mg (86%) of **16c,** mp 104–108 °C. Crystallization of the crude **16c** from ethyl acetate–hexane gave an analytical sample: mp 111–112 °C; IR (Nujol) 3240 (NH), 1730 and 1720 (C=O), 1660 and 1640 cm^{-1} (amide C=O, C=C); NMR (CD_3COCD_3) δ 8.13 (m, 2 H, ortho H's of C_6H_5), 7.67 (m, 3 H, meta and para H's of C_6H_5), 6.67 [d, 1 H, J = 10 Hz, $(CH_3)_2CHC\mathbf{H}$=], 3.76 (s, 3 H, OCH_3), 2.76 [m, 1 H, $(CH_3)_2C\mathbf{H}$-], 1.03 ppm [d, 6 H, J = 7 Hz, $(\mathbf{H}_3)_2CH$-].

Anal. Calcd for $C_{14}H_{17}NO_3$: C, 68.00; H, 6.93; N, 5.66. Found: C, 67.99; H, 6.96; N, 5.65.

α-Benzamido-β,β-dimethylacrylanilide (16e). A solution of 356 mg (0.92 mmol) of *N*-benzoyl-α-anilinovaline anilide (**17c**) in 10 ml of pyridine was refluxed for 24 h. The solvent was evaporated in vacuo and the crude residue was crystallized from ethanol, giving 233 mg (86%) of **16e:** mp 288–289 °C (lit.[12] mp 288–289 °C); IR (Nujol) 3300 and 3260 (NH), 1655 and 1635 cm^{-1} (amide C=O).

Methyl (*Z*)-α-Acetamidocinnamate (16a). A mixture of 630 mg (1.45 mmol) of **1g,** 1 g (7.24 mmol) of finely divided anhydrous potassium carbonate, and 0.3 ml (4.83 mmol) of methyl iodide in 8 ml of acetone-d_6 was stirred at room temperature for 2 days. The reaction mixture was filtered through Celite, and the solvent was evaporated in vacuo. The residue was separated by chromatography on a column (4.2 × 50 cm) of silica gel (60–200 mesh) by elution with chloroform giving 345 mg (86%) of tetrachlorocatechol dimethyl ether followed by 220 mg (69%) of crude **16a,** mp 110–122 °C. Crystallization twice from benzene gave 130 mg of pure **16a:** mp 127–127.5 °C (lit.[13] mp 125–126 °C); IR (Nujol) 3200 and 3150 (NH), 1725 (C=O), 1640 cm^{-1} (C=O).

2-(Phthalimidomethyl)-4-benzylidene-2-oxazolin-5-one (19). To a solution of 1.03 g (2.91 mmol) of phthaloylglycylphenylalanine (**18**) in 5 ml of acetic anhydride containing 1 drop of pyridine was added 651 mg (2.65 mmol) of *o*-chloranil, and after 2 h 0.5 ml of pyridine was added. After standing for 1 day, the reaction mixture was filtered, and the solid precipitate was dried in vacuo giving 851 mg (96%) of crude **19**, mp 215–220 °C. Crystallization from ethyl acetate–hexane gave 445 mg (50%) of an analytical sample of **19**: mp 232–233 °C; UV (95% ethanol) λ_{max} 333 nm (ϵ 22 500); IR (Nujol) 1810 and 1780 (C=O), 1665 cm^{-1} (C=N); NMR (Me_2SO-d_6), 90 MHz) δ 8.23–8.02 (m, 2 H, ortho H's of C_6H_5CH=), 7.94 (s, 4 H, phthaloyl H's), 7.48–7.36 (m, 3 H, meta and para H's of C_6H_5CH=), 7.33 (s, 1 H, C_6H_5CH=), and 4.90 ppm (s, 2 H, CH_2); ^{13}C (Me_2SO-d_6) 166.8, 166.3, and 163.3 (amide C=O, oxazolin C=O, C=N), 134.8, 132.7, 132.0, 131.4, 128.8, and 123.4 (aromatic and vinyl), 35.5 ppm (CH_2N); mass spectrum *m/e* (rel intensity) 332 (41), 188 (22), 161 (42), 160 (45), 133 (19), 116 (18), 104 (42), 89 (28), 77 (50), 76 (50), 63 (21), 51 (39), 50 (39), 39 (13).

Anal. Calcd for $C_{19}H_{12}N_2O_4$: C, 68.67; H, 3.64; N, 8.43. Found: C, 68.75; H, 3.64; N, 8.43.

***N*-Phthaloylglycinedehydrophenylalanine Ethyl Ester (20).** To a solution of 1 ml of 0.5 N sodium ethoxide in 25 ml of absolute ethanol, 200 mg (0.69 mmol) of **19** was added. After stirring for 30 min at room temperature the reaction mixture was poured into a flask containing 4 ml (7.0 mequiv) of Amberlite IR-120H resin in 4 ml of ethanol. After 30 min, the resin was removed by filtration and the solvent was evaporated in vacuo. The crude residue was crystallized from 2:1 ethanol–watr, giving 200 mg (88%) of **20**: mp 200–202 °C (lit.[6] 200–201 °C); IR (KBr) 3270, 1775, and 1730 (C=O), 1690 and 1650 cm^{-1} (amide C=O).

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Registry No.—**1a,** 60422-60-8; **1b,** 60422-61-9; **1c,** 60422-62-0; **1d,** 60422-63-1; **1e,** 60422-64-2; **1f,** 60422-65-3; **1g,** 60676-46-2; **2a,** 60422-66-4; **2b,** 60422-67-5; **2d,** 56538-58-0; **2e,** 60442-68-6; **3a,** 60422-70-0; **3b,** 60676-45-1; **4,** 60422-77-7; **5a,** 60676-48-4; **5b,** 60422-81-3; **5c,** 60422-82-4; **5d,** 60676-49-5; **6,** 60676-50-8; **7a,** 60422-83-5; **7c,** 60422-84-6; **9,** 7554-10-1; **12,** 60422-80-2; **16a,** 60676-51-9; **16b,** 26924-22-1; **16c,** 60676-52-0; **16d,** 27573-05-3; **16e,** 60676-53-1; **18,** 60676-54-2; **19,** 60676-55-3; **20,** 55424-41-4; $HO_2CCH(R)NHCOR'$ (R = $PhCH_2$; R' = Ph), 2901-76-0; $HO_2CCH(R)NHCOR'$ (R = $(CH_3)_2CH$; R' = Ph), 2901-80-6; $HO_2CCH(R)NHCOR'$ (R = CH_3; R' = Ph), 1205-02-3; $HO_2CCH(R)$-$NHCOR'$ (R = H; R' = Ph), 495-69-2; $HO_2CCH(R)NHCOR'$ (R = Ph; R' = Ph), 29670-63-1; $HO_2CCH(R)NHCOR'$ (R = $(CH_3)_2CHCH_2$; R = CH_3), 17966-67-5; *o*-chloranil, 2435-53-2; DL-phenylalanine, 150-30-1; aniline, 62-53-3; benzyl mercaptan, 100-53-8.

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Structure of Satratoxin H, a Metabolite of *Stachybotrys atra*. Application of Proton and Carbon-13 Nuclear Magnetic Resonance

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The structure of satratoxin H, a toxic metabolite of *Stachybotrys atra*, has been shown to be **4a** by spectroscopic studies. Satratoxin H is a macrocyclic dilactone derivative of the sesquiterpene 12,13-epoxytrichothec-9-ene and is structurally similar to roridin E, a known metabolite of *Myrothecium verrucaria*. It is most probably one of the causative agents of stachybotryotoxicosis, a food-borne disease which has affected both livestock and humans and which presents a potentially serious public health hazard.

Stachybotryotoxicosis is a food-borne disease which has affected both livestock and humans and which presents a potentially serious public health hazard.[1] The disease results from eating foods contaminated with toxic metabolites of the fungus *Stachybotrys atra*. Recent work in our laboratories has demonstrated that several derivatives of 12,13-epoxytrichothec-9-ene (**1a**) are produced by this mold and are most probably the cause of this disease.[2] Two of these metabolites, initially designated satratoxins C[3] and D,[2] were found by thin layer chromatography and ^1H NMR and mass spectral examination to be, in fact, the trichothecenes verrucarin J (**2**)[4] and roridin E (**3**),[5] respectively, reported by Tamm and co-