4-(Bromomethyl)-2-(methylthio)pyrimidine (26): yield 30%; an unstable oil; ^{1}H NMR δ 2.62 (s, Me), 4.40 (s, CH_2Br), 7.17 (d, J = 5 Hz, 5-H), 8.58 (d, J = 5 Hz, 6-H); HRMS m/z (M^+) calcd 217.9514, obsd 217.9521.

5-Bromo-4-(bromomethyl)-2-(methylthio)pyrimidine (27): yield 7%; mp 62-64 °C; ^{1}H NMR δ 2.60 (s, Me), 4.53 (s, CH_2Br), 8.63 (s, 6-H). Anal. Calcd for $C_6H_6Br_2N_2S$: C, 24.18; H, 2.03; N, 9.40. Found: C, 24.27; H, 2.02; N, 9.48.

Oxidation of 24 with Br_2 in AcOH-AcONa. A solution of **24** (0.33 g, 2.36 mmol) and sodium acetate (3.8 g) in acetic acid (8 mL) was heated at 70 °C and treated dropwise within 1 h with a solution of bromine (1.23 g, 7.67 mmol) in acetic acid (7 mL). After an additional 10 min the mixture was cooled, diluted with water (10 mL), neutralized with solid $NaHCO_3$, and then extracted with $CHCl_3$ (6 × 10 mL).[21] Chromatography on silica gel (CH_2Cl_2-AcOEt (8:2)) gave sulfone[28] **29** (0.065 g, 16%), which was eluted first, and sulfoxide **28** (0.20 g, 54%). Treatment of **28** with bromine under the same conditions gave **29**.

4-Methyl-2-(methylsulfinyl)pyrimidine (28): an oil; ^{1}H NMR δ 2.70 (s, 4-Me), 3.00 (s, S(O)Me), 7.42 (d, J = 5 Hz, 5-H), 8.82 (d, J = 5 Hz, 6-H). Anal. Calcd for $C_6H_8N_2OS$: C, 46.13; H, 5.16; N, 17.94. Found: C, 45.77; H, 5.19; N, 17.65.

4-Methyl-2-(methylsulfonyl)pyrimidine (29): mp 38-40 °C; ^{1}H NMR δ 2.73 (s, 4-Me), 3.42 (s, SO_2Me), 7.58 (d, J = 5 Hz, 5-H), 8.88 (d, J = 5 Hz, 6-H). Anal. Calcd for $C_6H_8N_2O_2S$: C, 41.84; H, 4.68; N, 16.27. Found: C, 41.54; H, 4.60; N, 15.98.

Reactions of (Bromomethyl)pyrimidines 12, 13, and 16 with DMSO. A solution of **12**, **13**, or **16** (1 mmol) in anhydrous DMSO (2 mL) was allowed to stand at 23 °C for 24 h and then treated with water (1 mL). The mixture was extracted with ether (5 × 10 mL), and the extract was dried (Na_2SO_4) and concentrated. The oxidation of **12** in the presence of $NaHCO_3$[18] (0.5 g) was conducted in a similar manner. Crude products were purified by chromatography.

4-Methyl-2-(methylthio)-5-pyrimidinecarbaldehyde (15): yield 63%; mp 67-69 °C (lit.[17] mp 63-64 °C); ^{1}H NMR spectrum was identical with that reported.[17]

5-Methyl-2-(methylthio)-4-pyrimidinecarbaldehyde (17): yield 78%; mp 56-57 °C; ^{1}H NMR δ 2.45 (s, 5-Me), 2.60 (s, SMe), 8.75 (s, 6-H), 9.93 (s, CHO); IR (neat) ν 1719 (C=O) cm^{-1}. Anal. Calcd for $C_7H_8N_2OS$: C, 49.98; H, 4.79; N, 16.66. Found: C, 50.07; H, 4.79; N, 16.58.

5-(Hydroxymethyl)-4-methyl-2-(methylthio)pyrimidine (18a): yield 36%; an oil; ^{1}H NMR δ 2.48 (s, 4-Me), 2.57 (s, SMe), 3.70 (br s, OH), 4.67 (s, CH_2), 8.38 (s, 6-H). Compound **18a** was transformed into a crystalline *p*-nitrobenzoyl derivative **18b** as described:[14] mp 149-150 °C (lit.[14] mp 148.5-149.5 °C).

5-(Hydroxymethyl)-2-(methylthio)-4-pyrimidinecarbaldehyde (19a) and 6-(Methylthio)-1,3-dihydrofuro[3,4-*d*]pyrimidin-1-ol (19b): yield 74%; mp 136-137 °C (lit.[19] mp 135-136 °C); IR (KBr) ν 3283 (OH) cm^{-1}; no C=O absorption; IR ($CHCl_3$) ν 1735 (C=O) cm^{-1}. The two forms exist in $CDCl_3$ solution in the ratio **19a**:**19b** = 1:3. ^{1}H NMR (400 Hz) for **19a**: δ 2.65 (s, SMe), 3.00 (t, J = 6 Hz, OH), 4.84 (d, J = 6 Hz, 5-CH_2), 8.80 (s, 6-H), 10.04 (s, CHO). ^{1}H NMR (400 Hz) for **19b**: δ 2.61 (s, SMe), 3.51 (d, J = 6 Hz, OH), 5.05 and 5.26 (2d, J = 13 Hz, 3a-CH_2), 6.33 (s, 7a-CH), 8.56 (s, 4-H).

Acknowledgment. We thank the donors of the Petroleum Research Fund, administered by the American Chemical Society, and the National Institutes of Health (Grant AI27196) for support of this research. The Varian VXR-400 NMR spectrometer was obtained with partial support from an award by the NSF Chemical Instrumental Program (CHEM-8409599).

(28) Sulfone **29** is mentioned in: Gefenas, V. *Proceedings of the Conference of Young Chemists*; Vilnius State University: Vilnius, USSR, 1980; p 87; *Chem. Abstr.* **1981**, *94*, 156861h).

Regioselective Functionalization of *N*-Phthaloyl-Substituted Amino Acid and Peptide Derivatives

Christopher J. Easton,* Craig A. Hutton, Giovanna Rositano, and Eng Wui Tan

Department of Organic Chemistry, University of Adelaide, G.P.O. Box 498, Adelaide, South Australia 5001

Received April 9, 1991

The free-radical reactions of a range of amino acid derivatives with *N*-bromosuccinimide are described. The products and relative rates of these reactions indicate that the α-position of an *N*-phthaloyl-substituted α-amino acid derivative is much less reactive than that of a corresponding *N*-acyl amino acid derivative toward hydrogen atom transfer. This is attributed to the proactive effects of acylamino and carboxyl substituents, in contrast to the counteractive effects of phthalimido and carboxyl groups. The reactions exemplify procedures for the regiocontrolled functionalization of amino acid and peptide derivatives.

Introduction

Hydrogen atom transfer reactions of *N*-acyl α-amino acid derivatives generally favor formation of α-carbon-centered radicals.[1] These radicals are resonance stabilized by the combined action of an electron-releasing amido substituent and an electron-withdrawing carboxy substituent, and they may be classified as captodative,[2] merostabilized,[3] or push-pull-stabilized[4] radicals. Amido-, carboxy-substituted radicals have been identified in proteins upon irradiation[5] and are thought to be intermediates in the photoalkylation[6] and carboxylation[7] of peptides.

(1) For a review of the formation of α-carbon-centered radicals in reactions of amino acid derivatives, see: Easton, C. J. *Advances in Detailed Reaction Mechanisms*; Coxon, J. M., Ed.; JAI Press: Greenwich, CT, 1991; Vol. 1, p 83.

(2) Viehe, H. G.; Merenyi, R.; Stella, L.; Janousek, Z. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 917. Viehe, H. G.; Janousek, Z.; Merenyi, R. *Acc. Chem. Res.* **1985**, *18*, 148.

(3) Baldock, R. W.; Hudson, P.; Katritzky, A. R.; Soti, F. *Heterocycles* **1973**, *1*, 67. Baldock, R. W.; Hudson, P.; Katritzky, A. R.; Soti, F. *J. Chem. Soc., Perkin Trans. 1* **1974**, 1422.

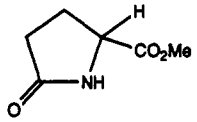
(4) Balaban, A. T. *Rev. Roum. Chim.* **1971**, *16*, 725.

(5) Henrikson, T.; Melo, T. B.; Saxebol, G. *Free Radicals in Biology*; Pryor, W. A., Ed.; Academic Press: New York, 1976; Vol. 2, p 213.

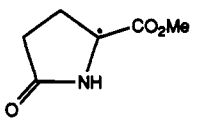
(6) Elad, D.; Sperling, J. *J. Chem. Soc. C* **1969**, 1579. Elad, D.; Schwarzberg, M.; Sperling, J. *J. Chem. Soc., Chem. Commun.* **1970**, 617. Sperling, J.; Elad, D. *J. Am. Chem. Soc.* **1971**, *93*, 967. Sperling, J.; Elad, D. *J. Am. Chem. Soc.* **1971**, *93*, 3839. Schwarzberg, M.; Sperling, J.; Elad, D. *J. Am. Chem. Soc.* **1973**, *95*, 6418.

0022-3263/91/1956-5614$02.50/0

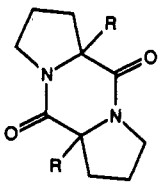
Other examples of their generation include the formation and dimerization of the radical **2** on irradiation of a mixture of methyl pyroglutamate (**1**) and di-*tert*-butyl peroxide,[8] and the oxidation of **3a** to give the diperoxide **3b**, presumably via **4a** and **4b**.[9]



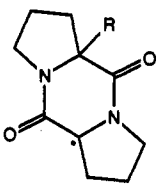
1



2



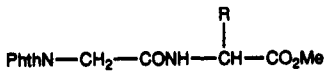
3



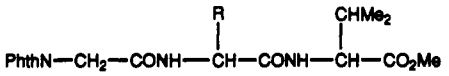
4

a) R = H
b) R = OOH

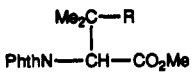
Consistent with this general trend, reactions of *N*-acyl α-amino acid derivatives with *N*-bromosuccinimide (NBS) proceed via formation of the corresponding α-carbon-centered radicals.[10,11] In direct contrast, however, formation of α-carbon-centered radicals from *N*-phthaloyl-substituted amino acid derivatives is strongly disfavored.[12] The extent of this effect is reflected in the regiospecific reactions of **5a–8a** to give the corresponding bromides **5b–8b**. In this report we describe the reactions of NBS with a range of amino acid derivatives, chosen to examine the cause of the contrasting effects of the *N*-phthaloyl and *N*-acyl substituents and to further illustrate the exploitation of those effects in the regioselective functionalization of amino acid and peptide derivatives.



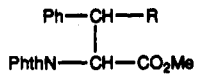
5



6



7



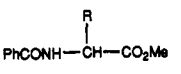
8

a) R = H
b) R = Br

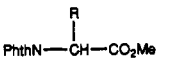
(7) Wheelan, P.; Kirsch, W. M.; Koch, T. H. *J. Org. Chem.* **1989**, *54*, 4360.
(8) Obata, N.; Niimura, K. *J. Chem. Soc., Chem. Commun.* **1977**, 238.
(9) Schmidt, U.; Hausler, J.; Ohler, E.; Poisel, H. *Prog. Chem. Org. Nat. Prod.* **1979**, *37*, 286.
(10) Easton, C. J.; Hay, M. P. *J. Chem. Soc., Chem. Commun.* **1985**, 425. Easton, C. J.; Hay, M. P.; Love, S. G. *J. Chem. Soc., Perkin Trans. 1* **1988**, 265.
(11) Easton, C. J.; Hay, M. P. *J. Chem. Soc., Chem. Commun.* **1986**, 55. Burgess, V. A.; Easton, C. J.; Hay, M. P. *J. Am. Chem. Soc.* **1989**, *111*, 1047.
(12) Easton, C. J.; Tan, E. W.; Hay, M. P. *J. Chem. Soc., Chem. Commun.* **1989**, 385.

Results and Discussion

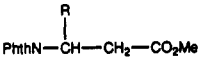
To investigate the contrasting effects of *N*-phthaloyl and *N*-acyl substituents on the formation of α-carbon-centered radicals in reactions of α-amino acid derivatives, reactions of the methyl esters of *N*-benzoylglycine (**9a**), *N*-phthaloylglycine (**10a**), *N*-phthaloyl-β-alanine (**11a**), and *N*-benzoyl-β-alanine (**12**) with NBS were investigated and compared. The β-alanine derivatives **11a** and **12**, which have the phthalimido and benzamido substituents spacially separated from the methoxycarbonyl group, were chosen to examine the relative effects of these substituents acting individually, while the glycine derivatives **9a** and **10a** were selected to study the effects of the benzamido and phthalimido substituents in combination with the methoxycarbonyl group.



9

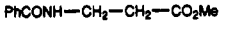


10

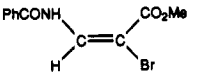


11

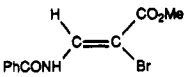
a) R = H
b) R = Br



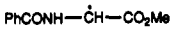
12



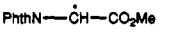
13



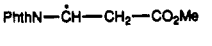
14



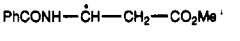
15



16



17



18

The reactions of **9a** and **11a** with NBS (1 equiv) in carbon tetrachloride gave the corresponding bromides **9b** and **11b**. Similar treatment of **12** gave a mixture of the α,β-dehydro-β-alanine derivatives **13** and **14** and unreacted starting material, while with excess NBS (2 equiv) all of the starting material was consumed. The reaction of **9a** was complete in 0.25 h, whereas the reactions of **11a** and **12** required 2 h for complete reaction. The reaction of **10a** to give **10b** was much less efficient, requiring excess NBS (2 equiv) and a reaction time of 48 h for 50% conversion. This qualitative comparison of the reactivity of **9a**, **10a**, **11a**, and **12** with NBS is reflected in the relative rates of reaction of the amino acid derivatives, which were determined in competitive experiments using, for example, equimolar mixtures of **9a** and **12**, **10a** and **11a**, and **11a** and **12**. Aliquots of crude reaction mixtures were easily and conveniently analyzed, by ^{1}H NMR spectroscopy, for consumption of starting materials and formation of products. In the competitive reaction of **9a** and **12** there was no evidence for any reaction of **12** until reaction of **9a** to give **9b** appeared to be complete. Similarly, with mixtures of **10a** and **11a**, and **11a** and **12**, **11a** reacted to the exclusion of **10a**, and **12** reacted to the exclusion of **11a**, within the limits of detection by ^{1}H NMR spectroscopy. From these experiments a conservative estimate for the relative rates of reaction of **9a** and **12**, **12** and **11a**, and **11a** and **10a** is 10:1 in each case.

The products of treatment of **9a**, **10a**, **11a**, and **12** with NBS indicate that the reactions involve formation of the corresponding radicals **15–18**, with subsequent bromine incorporation. In the reaction of **12**, subsequent elimination of hydrogen bromide, bromine addition, and hydrogen bromide elimination afford **13** and **14**. The regioselectivity of hydrogen atom transfer from **12** can be deduced from the greater relative rate of reaction of **12** compared to **11a** and the regioselectivity of reaction of **11a**. Reaction at the

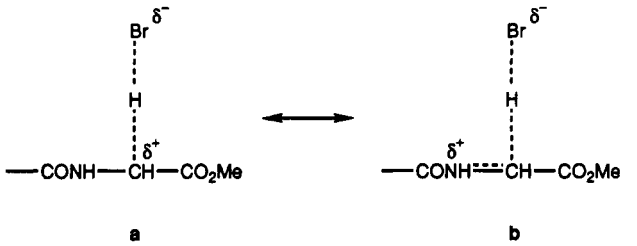


Figure 1. (a) Development of charge in the transition state for hydrogen atom abstraction by bromine atom and (b) delocalization of that charge by an amido substituent.

methylene adjacent to the methoxycarbonyl substituent in **12** would be inconsistent with the greater relative rate of reaction of **12** compared to **11a** and the reaction of **11a** at the methylene substituted with the phthalimido group in preference to reaction adjacent to the methoxycarbonyl substituent.

On this basis, the relative rates of reaction of **9a**, **10a**, **11a**, and **12** reflect the comparative ease of formation of the corresponding radicals **15–18**. The faster rate of reaction of **12** compared to **11a** can be attributed to the greater resonance stabilization provided by the benzamido substituent in **18** than by the phthalimido substituent in **17**. From the greater relative rate of formation of **15** compared to **18**, the effect of the methoxycarbonyl group in combination with the benzamido substituent is proactive, whereas the slower relative rate of formation of **16** compared to **17** shows that the effect of the methoxycarbonyl group in combination with the phthalimido substituent is counteractive. A radical formed adjacent to a methoxycarbonyl substituent is resonance stabilized, but formation of such a radical by hydrogen transfer to a bromine atom is often disfavored by a polar effect, involving the inductive interaction between the electron-deficient center of the substituent and that developing in the transition state at the site of hydrogen abstraction (Figure 1a).[13] The proactive nature of the methoxycarbonyl group in combination with the benzamido substituent may be attributed to the ability of the benzamido substituent to delocalize charge developed in the transition state (Figure 1b). The resulting diminution of the polar effect of the methoxycarbonyl substituent leads to the enhanced rate of formation of the resonance-stabilized radical **15**. The charge delocalization provided by the phthalimido substituent is less than that of the benzamido group, to the extent that this effect in the reaction of **10a** is outweighed by steric effects, resulting in the counteractive effect of the methoxycarbonyl group in combination with the phthalimido substituent. The steric effects will arise from interactions of the methoxycarbonyl and phthalimido substituents in **10a** with the hydrogen-abstracting species, and from interactions between the methoxycarbonyl and phthalimido substituents preventing the radical **16** from adopting planar conformations in which there is maximum delocalization of the unpaired spin density (Figure 2). These interactions will be greater than those between the methoxycarbonyl and benzamido substituents in **9a** and **15**.

In our initial report,[12] the contrasting effects of *N*-phthaloyl and *N*-acyl substituents were illustrated with

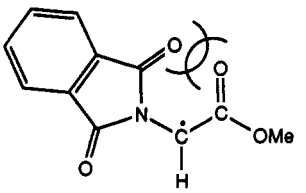
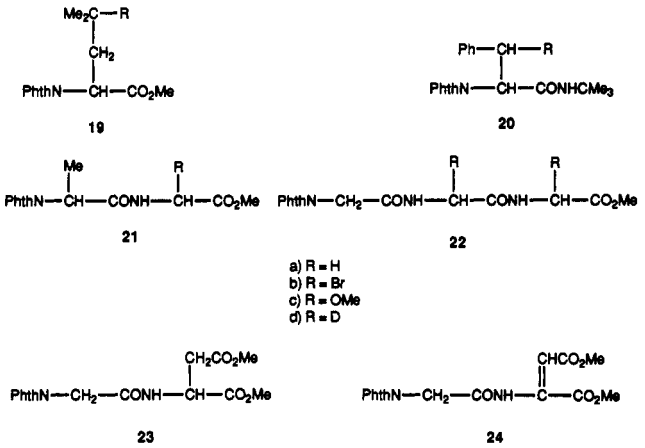


Figure 2. Nonbonding interactions associated with planar conformations of the radical **16**.

the reactions of **5a–8a** to give **5b–8b**, respectively. The reactions of **19a–22a** and **23** with NBS further demonstrate the extent of the counteractive effect of a carboxyl substituent in combination with the phthalimido substituent, to disfavor reaction at the α-position of amino acid derivatives. Treatment of the derivatives of leucine **19a** and phenylalaninamide **20a** with NBS (1 equiv) gave the corresponding bromides **19b** and **20b**, each in high yield. The reaction was repeated with the *S* isomer of **20a**, and the bromide **20b** thus obtained was treated with tributyltin hydride. The reduced product **20a** retained the homochirality of the starting material, as shown by comparison with a racemic sample using an HPLC column with (*S*)-phenylglycine as the chiral stationary phase.



The reaction of the peptide derivative **21a** with NBS (1 equiv) in dichloromethane gave the bromide **21b**, which was characterized by conversion to the methoxyglycine derivative **21c** and the deuteriated peptide derivative **21d**. Mass spectrometric analysis indicated that the deuterium incorporation of 90% in **21d** was regiospecific. The methoxyglycine derivative **21c** was obtained as a 1:1 mixture of diastereomers, as determined by ^{1}H NMR spectroscopy. The tripeptide derivative *N*-phthaloylglycylglycylglycine methyl ester (**22a**) was only sparingly soluble in dichloromethane, and its reaction with NBS had to be conducted in dilute solution. Under those conditions some of the NBS was consumed through reaction with the solvent. Thus, an excess of NBS (4 equiv) was required to produce the dibromide **22b**. Treatment of the crude dibromide **22b** with tributyltin deuteride gave **22d**, in 55% yield based on **22a**, with 90% and 95% deuterium incorporation at the C-terminal and nonterminal glycine residues, respectively. The reaction of *N*-phthaloylglycylaspartic acid dimethyl diester (**23**) with NBS gave an 82% yield of the α,β-dehydroaspartate derivative **24**, which was assumed to have *Z* stereochemistry on the basis of the tendency of dehydro amino acid derivatives to favor this configuration.[14]

In summary, the reactions of **5a–11a**, **12**, **19a–22a**, and **23** illustrate the contrasting effects of *N*-phthaloyl and

(13) Russell, G. A. *J. Am. Chem. Soc.* **1956**, *78*, 1047. Russell, G. A. *J. Org. Chem.* **1958**, *23*, 1407. Russell, G. A.; Williamson, R. C. *J. Am. Chem. Soc.* **1964**, *86*, 2357. Friedrich, S. S.; Friedrich, E. C.; Andrews, L. J.; Keefer, R. M. *J. Org. Chem.* **1969**, *34*, 900. Russell, G. A. *Free Radicals*; Kochi, J. K., Ed.; Wiley: New York, 1973; Vol. 1, p 275. Poutsma, M. L. *Free Radicals*; Kochi, J. K., Ed.; Wiley: New York, 1973; Vol. 2, p 159.

(14) Schmidt, U.; Lieberknecht, A.; Wild, J. *Synthesis* **1988**, 159 and references cited therein.

N-acyl substituents on the regioselectivity of reactions of amino acid and peptide derivatives with NBS. They exemplify procedures for the functionalization of amino acid derivatives and for the regiocontrolled halogenation of peptide derivatives.

Experimental Section

General. Melting points are uncorrected. ^{1}H NMR spectra were recorded as dilute solutions in deuteriochloroform on a Bruker CXP-300 spectrometer. Chemical shifts (δ) are reported in parts per million downfield from tetramethylsilane. Electron-impact mass spectra were recorded on an AEI MS-3010 spectrometer. Microanalyses were performed by the Canadian Microanalytical Service Ltd., New Westminster, British Columbia, Canada. HPLC was performed by using a Waters Model 501 solvent delivery system and a U6K injector with a Waters Model 481 absorbance detector. Preparative chromatography was carried out on a Chromatotron 7924T instrument (Harrison Research, Palo Alto/TC Research, Norwich, CA) by using Merck silica gel 60 PF_{254}, eluting with a gradient of light petroleum/dichloromethane/ethyl acetate. Light petroleum refers to the fraction with bp 55–65 °C. Carbon tetrachloride and dichloromethane were purified by fractional distillation and stored over 4A molecular sieves. NBS was recrystallized from water and dried under reduced pressure before use. A Philips MLU 300-W (220–240-V) ultraviolet lamp was used as the light source in reactions of NBS. Tributyltin deuteride was prepared by reduction of tributyltin chloride with lithium aluminum deuteride.[15] *N*-Benzoylglycine methyl ester (**9a**),[16] *N*-phthaloylglycine methyl ester (**10a**),[17] *N*-phthaloyl-β-alanine methyl ester (**11a**),[18] *N*-benzoyl-β-alanine methyl ester (**12**),[19] *N*-phthaloylleucine methyl ester (**19a**),[20] (*S*)- and (*RS*)-*N*-(*tert*-butyl)-*N*$^\alpha$-phthaloylphenylalaninamide (**20a**), *N*-phthaloylalanylglycine methyl ester (**21a**),[21] *N*-phthaloylglycylglycylglycine methyl ester (**22a**),[21] and *N*-phthaloylglycylaspartic acid dimethyl diester (**23**) were prepared by using standard procedures.

***N*-Benzoyl-α-bromoglycine Methyl Ester (9b).** A mixture of the glycine derivative **9a** (200 mg, 1 mmol) and NBS (180 mg, 1 mmol) in carbon tetrachloride (20 mL) was heated at reflux under nitrogen for 0.25 h, with reaction initiated by irradiation with a 300-W ultraviolet lamp; then the reaction mixture was cooled, filtered, and concentrated under reduced pressure to give crude **9b**;[22] ^{1}H NMR 3.93 (3 H, s), 6.65 (1 H, d, J = 8 Hz), 7.30–7.90 (6 H, m).

***N*-Phthaloyl-α-bromoglycine Methyl Ester (10b).** Treatment of **10a** with NBS as described above for the preparation of **9b**, except that excess NBS (2 equiv) was used and the reaction mixture was heated at reflux for 48 h, gave a mixture of **10a** and **10b** in a ratio of ca. 1:1. Chromatography of the mixture on silica gave **10b** in 27% yield: mp 116–118 °C; ^{1}H NMR 3.88 (3 H, s), 6.68 (1 H, s), 7.80–8.00 (4 H, m); MS m/e 240, 238, 218, 160; MS m/e 237.9508 (M^+ – COOMe). Calcd for $C_9H_5{}^{79}BrNO_2$: 237.9504.

***N*-Phthaloyl-β-bromo-β-alanine Methyl Ester (11b).** Treatment of **11a** with NBS as described above for the preparation of **9b**, except that the reaction mixture was heated at reflux for 2 h, gave **11b** in 84% yield: mp 95–97 °C; ^{1}H NMR 3.64 (1 H, dd, J = 7 and 17 Hz), 3.70 (3 H, s), 3.89 (1 H, dd, J = 8 and 17 Hz), 6.59 (1 H, dd, J = 7 and 8 Hz), 7.20–7.90 (4 H, m); MS m/e 313, 311, 282, 280, 254, 252, 240, 238, 232, 200, 173, 172, 160. Anal. Calcd for $C_{12}H_{10}NO_4$: C, 46.2; H, 3.2; N, 4.5. Found: C, 46.4; H, 3.2; N, 4.6.

Reaction of *N*-Benzoyl-β-alanine Methyl Ester (12) with NBS. Treatment of **12** with NBS as described above for the preparation of **9b**, except that the reaction mixture was heated at reflux for 2 h, gave an oil that contained unreacted starting material and the dehydro-β-alanine derivatives **13** and **14**, in a ratio of ca. 3:2:1, as determined by ^{1}H NMR spectroscopic analysis. The components were separated by chromatography of the mixture on silica, to give **13** and **14** in yields of 32% and 15%, respectively. The stereochemistry of **13** and **14** was assigned on the basis of an X-ray crystal structure determination.[23]

Methyl (*E*)-3-benzamido-2-bromoacrylate (13): mp 98–99 °C; ^{1}H NMR 3.89 (3 H, s), 7.40–7.90 (5 H, m), 8.38 (1 H, br d, J = 12 Hz), 8.60 (1 H, d, J = 12 Hz). Anal. Calcd for $C_{11}H_{10}BrNO_3$: C, 46.5; H, 3.5. Found: C, 46.5; H, 3.5.

Methyl (*Z*)-3-benzamido-2-bromoacrylate (14): mp 122.5–123.5 °C; ^{1}H NMR 3.80 (3 H, s), 7.40–7.90 (5 H, m), 8.20 (1 H, d, J = 12 Hz), 11.30 (1 H, br d, J = 12 Hz). Anal. Calcd for $C_{11}H_{10}BrNO_3$: C, 46.5; H, 3.5. Found: C, 46.5; H, 3.7.

***N*-Phthaloyl-γ-bromoleucine Methyl Ester (19b).** Treatment of **19a** with NBS as described above for the preparation of **9b** gave **19b** in 82% yield: mp 63–64 °C; ^{1}H NMR 1.76 (3 H, s), 1.84 (3 H, s), 2.85 (2 H, m), 3.74 (3 H, s), 5.25 (1 H, dd, J = 4.5 and 8 Hz), 7.75–7.90 (4 H, m); MS m/e 296, 294, 274, 214. Anal. Calcd for $C_{15}H_{16}BrNO_4$: C, 50.8; H, 4.6; N, 4.0. Found: C, 50.9; H, 4.6; N, 4.0.

***N*-*tert*-Butyl-*N*$^\alpha$-phthaloyl-β-bromophenylalaninamide (20b).** Treatment of (*RS*)-**20a** with NBS as described above for the preparation of **9b** gave **20b** as a 1:1 mixture of diastereomers, which were separated by fractional crystallization from 2-propanol/light petroleum. One diastereomer was obtained as needle-shaped crystals in 41% yield: mp 199–200 °C; ^{1}H NMR 1.03 (9 H, s), 5.28 (1 H, d, J = 12 Hz), 5.85 (1 H, br s), 6.25 (1 H, d, J = 12 Hz), 7.30–8.00 (9 H, m); MS m/e 430, 428. Anal. Calcd for $C_{21}H_{21}BrN_2O_3$: C, 58.8; H, 4.9; N, 6.5. Found: C, 58.8; H, 4.9; N, 6.6. The other diastereomer was isolated as pale-yellow granular crystals in 39% yield: mp 213–213.5 °C; ^{1}H NMR 1.43 (9 H, s), 5.32 (1 H, d, J = 11.5 Hz), 6.04 (1 H, d, J = 11.5 Hz), 6.50 (1 H, br s), 7.20–7.80 (9 H, m); MS m/e 430, 428. Anal. Calcd for $C_{21}H_{21}BrN_2O_3$: C, 58.8; H, 4.9; N, 6.5. Found: C, 58.5; H, 5.0; N, 6.8.

Reaction of (*S*)-*N*-*tert*-Butyl-*N*$^\alpha$-phthaloylphenylalaninamide (20a) with NBS and Tributyltin Hydride. Treatment of (*S*)-**20a** with NBS as described above for the reaction of (*RS*)-**20a** gave a 1:1 mixture of the diastereomers of the bromide (**20b**). A sample of this mixture of the bromides (**20b**) (100 mg, 0.23 mmol) was treated with tributyltin hydride (67 mg, 0.23 mmol) in benzene (5 mL), at reflux under nitrogen for 4 h. The product (**20a**) (71 mg, 88%) showed only one component, with a retention time of 21 min, on HPLC analysis using a Regis Pirkle covalent (*S*)-phenylglycine column (25 cm × 4.6 nm), eluting with a gradient of light petroleum/2-propanol (1 mL/min, 5–40% over 40 min). By comparison, under the same conditions (*RS*)-**20a** resolved into two components, with retention times of 21 and 22 min.

***N*-Phthaloylalanyl-α-methoxyglycine Methyl Ester (21c).** The peptide derivative **21a** (0.7 mmol) was treated with NBS (125 mg, 0.7 mmol) in dichloromethane (25 mL), at reflux under nitrogen for 0.5 h, with reaction initiated by irradiation with a 300-W ultraviolet lamp. The reaction mixture was cooled and filtered, and the filtrate was concentrated under reduced pressure to give the crude bromide **21b** (^{1}H NMR 6.56, d, J = 10 Hz). Alternatively, methanol (0.5 mL) was added to the filtrate and the mixture was stirred at room temperature for 2 h; then it was concentrated under reduced pressure. The residual oil was chromatographed on silica and crystallized from dichloromethane/light petroleum to give the methoxyglycine derivative **21c** in 70% yield: mp 134–139 °C; ^{1}H NMR 1.72 (1.5 H, d, J = 7.5 Hz), 1.74 (1.5 H, d, J = 7.5 Hz), 3.45 (1.5 H, s), 3.47 (1.5 H, s), 3.78 (1.5 H, s), 3.79 (1.5 H, s), 5.00 (1 H, m), 5.56 (0.5 H, d, J = 9 Hz), 5.58 (0.5 H, d, J = 9 Hz), 6.93 (0.5 H, br d, J = 9 Hz), 6.98 (0.5 H, br d, J = 9 Hz), 7.70–7.90 (4 H, m); MS m/e 261, 202, 175, 174. Anal. Calcd for $C_{15}H_{16}N_2O_6$: C, 56.3; H, 5.0; N, 8.7. Found: C, 56.4; H, 5.0; N, 8.7.

***N*-Phthaloylalanyl-α-deuterioglycine Methyl Ester (21d).** Tributyltin deuteride (310 mg, 1.07 mmol) was added to a crude,

(15) Kuivila, H. G.; Beumel, O. F. *J. Am. Chem. Soc.* **1961**, *83*, 1246.
(16) Huang, H. T.; Niemann, C. *J. Am. Chem. Soc.* **1952**, *74*, 4634.
(17) Skorna, G.; Ugi, I. *Chem. Ber.* **1979**, *112*, 776.
(18) Clarke, S.; Hider, R. C.; John, D. I. *J. Chem. Soc., Perkin Trans. 1* **1973**, 230.
(19) Garapon, J.; Touet, R.; Sillion, B. Ger. Offen. DE 3,327,285, 1984.
(20) Tascher, E.; Wasielewski, C. *Justus Liebigs Ann. Chem.* **1961**, *640*, 136.
(21) Islam, A. M.; El-Naggar, A. M.; Abd-El-Salam, A. M.; Zaher, M. R. *Egypt. J. Chem.* **1975**, *18*, 35.
(22) Kober, R.; Papadopoulos, K.; Miltz, W.; Enders, D.; Steglich, W.; Reuter, H.; Puff, H. *Tetrahedron* **1985**, *41*, 1693.
(23) Easton, C. J.; Rositano, G.; Tiekink, E. R. T. *Acta Crystallogr.* **1990**, *C46*, 1122.

filtered reaction mixture of the bromide **21b**, prepared from **21a** (0.7 mmol) as described above. The mixture was stirred at room temperature for 2 h; then it was concentrated under reduced pressure. The residual oil was chromatographed on silica and crystallized from dichloromethane/light petroleum to give **21d** in 70% yield: mp 145–146 °C; ^{1}H NMR 1.73 (3 H, d, J = 7.5 Hz), 3.73 (3 H, s), 4.05 (1.1 H, d, J = 5 Hz), 4.98 (1 H, q, J = 7.5 Hz), 6.71 (1 H, br d, J = 5 Hz), 7.70–7.85 (4 H, m); MS m/e 291 (90% $^2H_1$), 232 (90% $^2H_1$), 202 (0% $^2H_1$).

***N*-Phthaloylglycyl-α-deuterioglycyl-α-deuterioglycine Methyl Ester (22d).** Addition of tributyltin deuteride (530 mg, 1.8 mmol) to a crude, filtered reaction mixture of **22b**, prepared from **22a** (200 mg, 0.6 mmol) and NBS (430 mg, 2.4 mmol) in dichloromethane (200 mL), as described above for the preparation of **21b**, gave **22d** (0.11 g, 55%): mp 230–231 °C; ^{1}H NMR 3.69 (3 H, s), 3.86 (1.1 H, d, J = 5 Hz), 3.90 (1.05 H, d, J = 6 Hz), 4.37 (2 H, s), 7.70–7.90 (4 H, m), 8.14 (1 H, br d, J = 6 Hz), 8.51 (1 H, br d, J = 5 Hz); MS m/e 335 (90% $^2H_2$, 5% $^2H_1$), 246 (95% $^2H_1$), 218 (95% $^2H_1$), 188 (0% $^2H_1$).

***N*-Phthaloylglycyl-(*Z*)-α,β-dehydroaspartic Acid Dimethyl Diester (24).** Treatment of **23** (400 mg, 1.15 mmol) with NBS (205 mg, 1.15 mmol), as described above for the preparation of **21b**, gave **24** (330 mg, 82%): mp 175–176 °C; ^{1}H NMR 3.73 (3 H, s), 3.79 (3 H, s), 4.50 (2 H, s), 5.57 (1 H, s), 7.70–7.90 (4 H, m), 10.50 (1 H, br s); MS m/e 346, 345, 314, 287, 188, 161, 160. Anal. Calcd for $C_{16}H_{14}N_2O_7$: C, 55.5; H, 4.1; N, 8.1. Found: C, 55.5; H, 4.0; N, 8.1.

Acknowledgment. This work was supported by a grant from the Australian Research Council.

Registry No. **9a**, 1205-08-9; **9b**, 101649-82-5; **10a**, 23244-58-8; **10b**, 135395-15-2; **11a**, 39739-01-0; **11b**, 135395-26-5; **12**, 89928-06-3; **13**, 129309-14-4; **14**, 129309-13-3; **19a**, 132785-19-4; **19b**, 132785-25-2; (*S*)-**20a**, 135395-13-0; (*RS*)-**20a**, 135501-56-3; (2*S*,3*R*)-**20b**, 135395-16-3; (2*S*,3*S*)-**20b**, 135395-17-4; (±)-(*R**,*R**)-**20b**, 135501-57-4; (±)-(*R**,*S**)-**20b**, 135501-58-5; **21a**, 63267-72-1; **21b** (diastereomer 1), 135395-18-5; **21b** (diastereomer 2), 135395-19-6; **21c** (diastereomer 1), 135395-20-9; **21c** (diastereomer 2), 135395-21-0; **21d** (diastereomer 1), 135395-22-1; **21d** (diastereomer 2), 135395-23-2; **22a**, 63199-92-8; **22b**, 135395-24-3; **22d**, 135395-25-4; **23**, 135395-14-1; **24**, 87358-90-5.

Variculanol: Structure and Absolute Stereochemistry of a Novel 5/12/5 Tricyclic Sesterterpenoid from *Aspergillus variecolor*

Sheo Bux Singh,* R. A. Reamer, D. Zink, D. Schmatz, A. Dombrowski, and M. A. Goetz

Merck Sharp & Dohme Research Laboratories, P.O. Box 2000, Rahway, New Jersey 07065

Received March 20, 1991

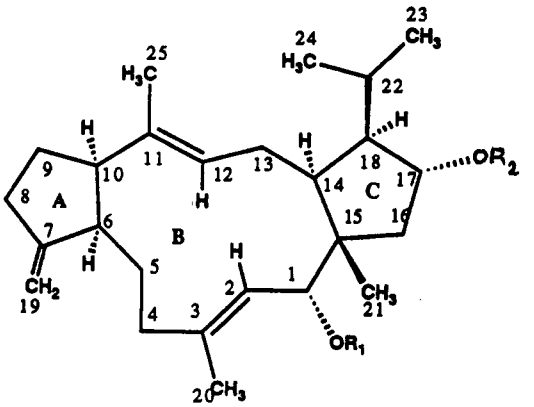
The structure, absolute stereochemistry, and conformation of variculanol, a sesterterpenoid with novel skeleton isolated from *Aspergillus variecolor*, has been described. The molecular structure was deduced from extensive application of 2D NMR methods, in particular HMBC, which established the unambiguous assignment of the novel 5/12/5 ring system. NOEDS measurements were very useful in establishing the relative stereochemistry. The NMR-mandelate method and CD spectral analysis of the 1,17-bis(4-bromobenzoate) were used to determine the absolute stereochemistry. A unique solution-phase conformation was determined from both extensive NOE measurements and MM2 calculations and optimization.

In our search for novel antiparasitic compounds effective against coccidia,[1] we discovered variculanol (**1a**), a sesterterpenoid having a novel 5/12/5 ring system from *Aspergillus variecolor*. We report herein the isolation, structure, absolute stereochemistry, and solution conformation of variculanol (**1a**).

A methyl ethyl ketone extract of a solid-state fermentation of *A. variecolor* was partitioned between hexane and methanol–water. Silica gel chromatography of the hexane extract followed by crystallization from CH_3CN afforded granules of variculanol[2] (**1a**).

Structure Elucidation. High-resolution EI mass spectral analysis of variculanol (**1a**) gave the molecular formula $C_{25}H_{40}O_2$ with six double-bond equivalents (DBE), which was corroborated by ^{13}C NMR spectral data (Table I). The IR spectrum of **1a** showed hydroxy absorption, which was confirmed by formation of a diacetate (**1b**, M^+, 456; IR 1733 cm^{-1}) and a bis(trimethylsilyl ether) (M^+, 516.3809). Because of the presence of only three double bonds, this molecule must have a tricyclic skeleton.

The 400-MHz ^{1}H NMR spectrum of **1a** in $CDCl_3$ and DMSO-d_6 (Table I) exhibited some readily assignable signals such as two vinylic methyls, an angular methyl and a set of methyl doublets, two oxymethines, two exocyclic methylene protons, and a pair of olefinic protons. The



1a: $R_1 = R_2 =$ H, variculanol
b: $R_1 = R_2 =$ acetyl
c: $R_1 =$ (*R*)-*O*-methylmandeloyl, $R_2 =$ H
d: $R_1 =$ H, $R_2 =$ (*R*)-*O*-methylmandeloyl
e: $R_1 =$ (*S*)-*O*-methylmandeloyl, $R_2 =$ H
f: $R_1 =$ H, $R_2 =$ (*S*)-*O*-methylmandeloyl
g: $R_1 =$ 4-bromobenzoyl, $R_2 =$ H
h: $R_1 =$ H, $R_2 =$ 4-bromobenzoyl
i: $R_1 = R_2 =$ 4-bromobenzoyl

(1) Schmatz, D. M.; Crane, M. J. S.; Murray, P. K. *J. Protozool.* **1986**, *33*, 109.
(2) Repeated efforts toward crystallization of variculanol and its derivatives failed to produce crystals suitable for X-ray diffraction.

0022-3263/91/1956-5618$02.50/0