and a 1-h reaction time was recrystallized from MeOH to give a pure **6b** (112 mg, 90%) as colorless plates: mp 203–206 °C (lit.[25] mp 205–207 °C); ^{1}H NMR ($CDCl_3$) δ 0.97 (3 H, s, 18-CH_3), 4.40 (1 H, m, 16β-H), 6.51–7.20 (3 H, m, aromatic protons).

Sodium 3β,16α-Dihydroxy-17-oxo-5-androsten-3-yl Sulfate (7). The 16α-bromo 17-ketone **1a** (2 g, 4.88 mmol) in 10 mL of dry pyridine was added to 1.5 equiv of pyridine–$ClSO_3H$ complex in 20 mL of pyridine with stirring under ice cooling. After 20 min the reaction mixture was poured into 1 L of chilled 0.1 N NaOH solution and allowed to stand at 0 °C for 3 h. The solution was passed through a column of Amberlite XAD-2 (4 × 100 cm). After the column was washed with water (1 L), the sulfate was eluted with MeOH (1 L). The eluate was condensed to 20 mL and allowed to stand at 4 C for 24 h. The precipitates (1.95 g) were collected by filtration and recrystallized from MeOH–Et_2O to give **7** (1.63 g, 85%) as colorless needles: mp > 280 °C; IR (KBr) ν_{max} 3440, 1738, 1235 cm^{-1}; ^{1}H NMR [pyridine-d_5–CD_3OD (1:3)] δ 0.92 (3 H, s, 18-CH_3), 0.97 (3 H, s, 19-CH_3), 4.19–4.70 (2 H, m, 3α-H and 16β-H). Anal. Calcd for $C_{19}H_{27}O_6SNa\cdot H_2O$: C, 53.76; H, 6.89; S, 7.55. Found: C, 53.54; H, 6.83; S, 7.42.

Hydrolysis of 16α-Bromo Ketone 1a with the NaOH–$H_2{}^{18}O$–Pyridine System. Bromo ketone **1a** or ketol **3a** (0.11 mmol) in 0.75 mL of pyridine was treated with NaOH–$H_2{}^{18}O$ [5 mg (0.125 mmol) of NaOH in 0.25 mL (13.89 mmol) of 99.5 atom % [^{18}O]-water; theoretical ^{18}O content of the solution 98.6 atom %] for 8 h at room temperature (condition F, Table II). The reaction mixture was poured into 5% HCl solution and extracted with AcOEt (2 × 20 mL). The organic layer was washed with 5% $NaHCO_3$ and water and dried with Na_2SO_4. After evaporation of the solvent, the residue (30–32 mg) was acetylated with pyridine (1 mL)–Ac_2O (0.5 mL). The crude acetates obtained by evaporation of the solvents under the reduced pressure were purified by TLC (*n*-hexane–AcOEt, 4:1) to give the pure **3a** diacetate [mp 167–168 °C (lit.[8] mp 167–168 °C); ^{1}H NMR ($CDCl_3$) δ 1.00 (3 H, s, 18-CH_3), 1.06 (3 H, s, 19-CH_3), 2.00 (3 H, s, 3β-$OCOCH_3$), 2.12 (3 H, s, 17β-$OCOCH_3$), 4.60 (1 H, br m, 3α-H), 5.43 (2 H, m, 6-H and 16β-H)] and the acetates of the recovered bromo ketones **1a** and **2a** (16α-Br/16β-Br ratio of 1:1.25).

Ketol Rearrangement of 3a. The ketol **3a** (25 mg, 0.082 mmol) in 3.5 mL of MeOH was treated with NaOH–$H_2{}^{18}O$ (7 mg of NaOH in 0.19 mL of 99.5 atom % [^{18}O]water) for 3 days at room temperature. The reaction mixture was poured into 5% HCl solution and then extracted with AcOEt (2 × 10 mL). The organic layer was washed with 5% $NaHCO_3$ and water and dried with Na_2SO_4. After evaporation of the solvent, the rearranged product was recrystallized from MeOH to give **4** (13 mg, 52%) as colorless needles: mp 204–206 °C (lit.[26] mp 202–205 °C); ^{1}H NMR ($CDCl_3$) δ 0.78 (3 H, s, 18-CH_3), 1.05 (3 H, s, 19-CH_3), 3.45 (1 H, br m, 3α-H), 3.73 (1 H, s, 17α-H), 5.43 (1 H, m, 6-H). **4** was acetylated with pyridine–Ac_2O as usual to give the **4** diacetate: mp 125 °C; ^{1}H NMR ($CDCl_3$) δ 0.83 (3 H, s, 18-CH_3), 1.06 (3 H, s, 19-CH_3), 2.00 (3 H, s, 3β-$OCOCH_3$), 2.13 (3 H, s, 17β-$OCOCH_3$), 4.66 (1 H, br m, 3α-H), 5.00 (1 H, s, 17α-H), 5.40 (1 H, m, 6-H).

16β-Morpholino-3β-hydroxy-5-androsten-17-one (8a). A solution of **1a** or **2a** (300 mg, 0.86 mmol) in morpholine (3 mL) was heated under reflux for 2 h. After removal of the majority of the amine by distillation under reduced pressure, the products were precipitated with water, and the gummy solid was washed with water. The crude 16β-morpholino 17-ketone was recrystallized from MeOH to give a pure **8a** as colorless plates: mp 201–202 °C (lit.[5a] mp 200 °C); ^{1}H NMR ($CDCl_3$) δ 0.90 (3 H, s, 18-CH_3), 1.03 (3 H, s, 19-CH_3), 2.67 (4 H, t, J = 5 Hz, 3′-H), 2.98 (1 H, q, J = 4, 2 Hz, 16α-H), 3.55 (1 H, br m, 3α-H), 3.90 (4 H, t, J = 5 Hz, 2′-H), 5.40 (1 H, m, 6-H).

16β-Morpholino-3β-hydroxy-5α-androstan-17-one (8b). A solution of **1b** (300 mg, 0.86 mmol) in morpholine was heated under reflux for 1 h. After the same workup as above, the crude 16β-morpholino compound was recrystallized from aqueous MeOH to give a pure **8b** as colorless needles: mp 195–198 °C (lit.[5a] mp 192–197 °C); ^{1}H NMR ($CDCl_3$) δ 0.83 (3 H, s, 19-CH_3), 0.86 (3 H, s, 18-CH_3), 2.67 (4 H, t, J = 5 Hz, 3′-H), 2.98 (1 H, q, J = 4, 2 Hz, 16α-H), 3.50 (1 H, m, 3α-H), 3.72 (4 H, t, J = 5 Hz, 2′-H).

Epimerization of 16β-Morpholino Derivative 8b. The 16β-morpholino derivative **8b** (90 mg, 0.24 mmol) was heated under reflux in morpholine (1 mL)–D_2O (0.5 mL) for 1 h. The solvent was evaporated under reduced pressure to give deuterated **8b**. After crystallization from MeOH–water, **8b**-*d* was isolated: 65% yield; mp 193–194 °C (lit.[5a] mp 192–197 °C).

Acknowledgment. We thank Mrs. Carol Yarborough for her able technical assistance. Research was supported in part by Grant HD-04945 from the National Institute of Child Health and Human Development, DHHS, Grant RR-05716 from the Division of Research Resources, DHHS, and a Research Grant from the Japan Association of Chemistry.

Registry No. **1a**, 1093-91-0; **1a** acetate, 24335-49-7; **1b**, 28507-02-0; **1b-16-d**, 82865-80-3; **2a**, 74644-60-3; **2a** acetate, 24335-50-0; **3a**, 1232-73-1; **3a** diacetate, 10587-75-4; **3b**, 10459-27-5; **4**, 1159-66-6; **4** diacetate, 1249-72-5; **5a**, 61145-69-5; **5b**, 63-02-5; **6a**, 71765-95-2; **6b**, 566-76-7; **7**, 35420-26-9; **8a**, 5986-91-4; **8b**, 3000-34-8.

(25) Biggerstaff, W. R. Gallagher, T. F. *J. Org. Chem.* **1957**, *22*, 1220.

(26) Kincl, F. A. *J. Steroid Biochem.* **1976**, *7*, 419.

An Excursion into the Synthesis of Potential Angiotensin Converting Enzyme Inhibitors

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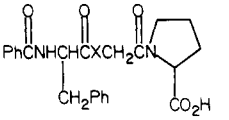
An attempt to prepare dehydro analogues of **1** gave the expected tripeptide **2a**, but rearrangement of a thiazolone derivative of Δ^Z-Phe made only a thiazolinecarboxylic acid (**8**) available. The latter was also converted into a tripeptide (**11**) and both compounds, **2a** and **11**, showed moderate angiotensin converting enzyme inhibition.

Angiotensin converting enzyme (ACE) is responsible for the hydrolysis[1] of a decapeptide, angiotensin I, to give the potent pressor octapeptide, angiotension II. Inhibition of this process allows control of hypertension in some human subjects. Recently reported ACE inhibitors include the simple tripeptide,[2a] *N*-Bz-Phe-Gly-Pro-OH (**1a**), which



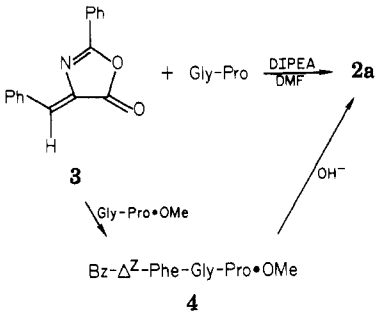
1a, X = NH
b, X = CH_2

shows an IC_{50}[3] for ACE of 9.4 × 10^{-6} M, and its "keto methylene" modification[2a] (**1b**), which has an IC_{50} of 7 ×

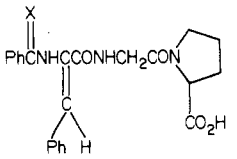
(1) "Handbook of Experimental Pharmacology"; Page, I. H., Bumpus, F. M., Eds.; Springer-Verlag: New York, 1974; Vol. 37.

0022-3263/82/1947-4029$01.25/0

Scheme I

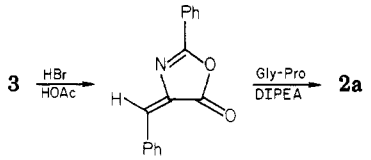


10^{-8} M. Since we have shown[4] that dehydroamino acid residues confer enzyme stability on some peptides, we have investigated the synthesis of the dehydro analogues of **1a**, **2a** and **2b**, as potential ACE inhibitors, expecting that the thio compound **2b** would bind more tightly to the enzyme active site than **2a** since it is well-known that ACE is a zinc-containing enzyme.[2e,5]



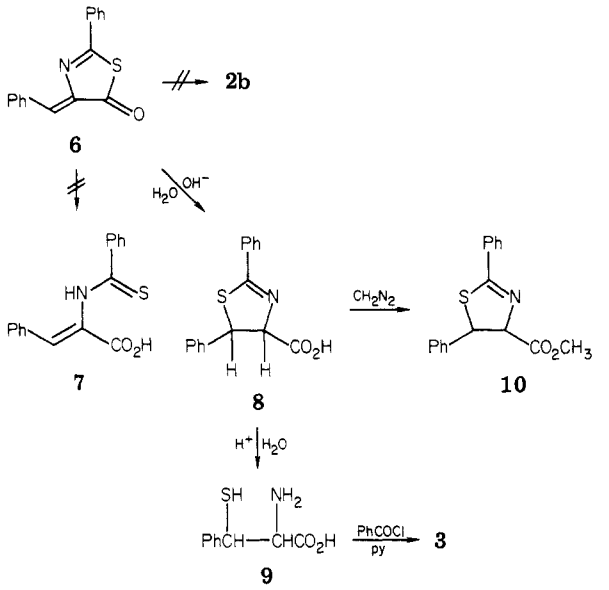
2a, X = O
b, X = S

The synthesis of **2a**, as shown in Scheme I, was essentially uneventful, since the direct condensation of the diisopropylethylamine (DIPEA) salt of Gly-Pro with the oxazolone **3** afforded **2a** in 65% yield. Condensation of **3** with the dipeptide ester gave the tripeptide ester **4** in reasonable yield (57%), but subsequent ester hydrolysis yielded pure **2a** in only 10% yield. It is noteworthy that two doublets (δ 4.17 and 3.94) assignable to the methylene protons of the glycine residue appeared in the NMR spectrum of **2a**. Since it is well-known[6] that amides at proline nitrogen often exist as mixtures of s-trans and s-cis conformations, these doublets were assigned to those conformers. Attempts were also made to prepare the geometric isomer of **2a**, Bz-Δ^E-Phe-Gly-Pro-OH.[7] The (*Z*)-oxazolone **3** was isomerized[8] to the *E* compound **5** with concentrated HBr and **5** was then condensed with the



5

Scheme II



dipeptide salt as before. Only the *Z* tripeptide **2a** was obtained. Other reaction conditions were tried, but the *Z* isomer was the only product observed. Consistent with previous work,[8] TLC analysis of the reaction mixtures indicated that the (*E*)-oxazolone was being isomerized by the basic conditions of the reaction before condensation occurred.

The synthesis of the thio analogue, **2b**, was envisioned as proceeding through the thiooxazolone (**6**), available from **3** by treatment with thiolacetic acid.[10] In fact, treatment of **6** with the dipeptide salt under various conditions gave an intractable mixture of products containing very little (TLC) of the desired compound. An alternative synthesis of **2b** using the thiamido acid **7** was then planned. Since oxazolone rings are readily hydrolyzed to give the corresponding dehydroamino acid derivatives, basic hydrolysis of **6** was expected to afford acid **7**. The melting point of our hydrolysis product agreed with that reported[10] and elemental analysis confirmed the empirical formula as $C_{16}H_{13}NO_2S$. In the NMR, a two-proton singlet at δ 5.53 ($CDCl_3$) that became two doublets (AB) at δ 5.50 and 5.43 in Me_2SO-d_6/$CDCl_3$ was, however, inconsistent with the structure **7**. The absence of an NH stretching absorption in the infrared spectrum and the presence of two ^{13}C singlets (86.078 and 56.145 ppm), which in the coupled spectrum appear as doublets, convinced us to assign the thiazoline structure **8** to this product (Scheme II). Hot acid hydrolysis of **8** gave a crude product that was both ninhydrin (NH_2) and nitroprusside (SH) positive and was assigned the β-mercaptophenylalanine structure **9**. Confirmation of this came when **9** was converted to **3** upon treatment with excess benzoyl chloride in pyridine.[11] Esterification of **8** (diazomethane) gave a methyl ester (**10**) that showed the H-4 and H-5 atoms clearly in the NMR spectrum, again confirming the thiazoline structure. Apparently **8** is formed from **6** by cyclization of the amide anion (**12**) formed when **6** reacts with alkali. This reaction has no precedent in the oxazolone series, but there is an early example of a similar rearrangement.[12] Surprisingly,

(2) (a) Almquist, R. G.; Chao, W-R.; Ellis, M. E.; Johnson, H. L. *J. Med. Chem.* **1980**, *23*, 1392. (b) Meyer, R. F.; Nicolaides, E. D.; Tinney, F. J.; Lunney, E. A.; Holmes, A.; Hoefle, M. L.; Smith, R. D.; Essenburg, A. D.; Kaplan, H. R.; Almquist, R. G. *Ibid.* **1981**, *24*, 964. (c) Patchett, A. A. et al. *Nature (London)* **1980**, *288*, 280. (d) Konno, S.; Stammer, C. H. *Int. J. Pept. Protein Res.* **1978**, *12*, 222. (e) Ondetti, M. A.; Rubin, B.; Cushman, D. W. *Science* **1977**, *196*, 441.

(3) Molar concentration that shows 50% inhibition.

(4) (a) English, M. L.; Stammer, C. H. *Biochem. Biophys. Res. Commun.* **1978**, *85*, 780. (b) Shimohigashi, Y.; Chen, H-C.; Stammer, C. H. *Ibid.*, in press.

(5) Ondetti, M. A.; Williams, N. J.; Sabo, E. F.; Plascec, J.; Weaver, E. R.; Kocy, O. *Biochemistry* **1971**, *10*, 4033.

(6) Galary, R. E.; Alger, J. R.; Liakopoulou-Kyriakides, M. *Int. J. Pept. Protein Res.* **1982**, *19*, 123–132.

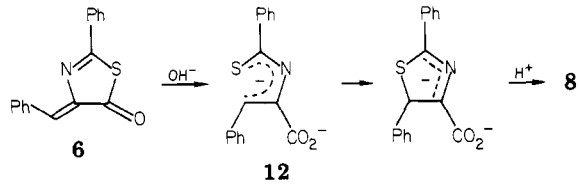
(7) The symbols Δ^Z-Phe and Δ^E-Phe represent the *Z* and *E* stereoisomers of dehydrophenylalanine.

(8) Tatsuoka, S.; Morimoto, A. *Yakugaki Zasshi* **1950**, *70*, 2531; *Chem. Abstr.* **1951**, *45*, 1541.

(9) (a) Carter, H. E.; Risser, W. C. *J. Biol. Chem.* **1941**, *139*, 255. (b) Rao, Y. S.; Filler, R. *Synthesis* **1975**, 749. (c) Boyd, G. V.; Wright, P. H. *J. Chem. Soc., Perkin Trans 1* **1972**, 909.

(10) Layre, S. I.; Gatenko, L. G. *J. Gen. Chem. USSR* **1952**, *22*, 321.

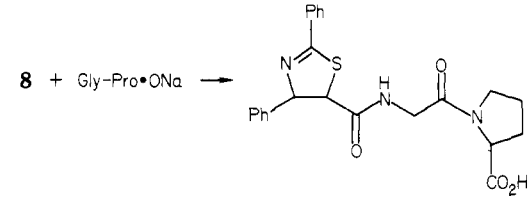
(11) β-Hydroxyphenylalanine gives **3** under exactly the same conditions.



6 12

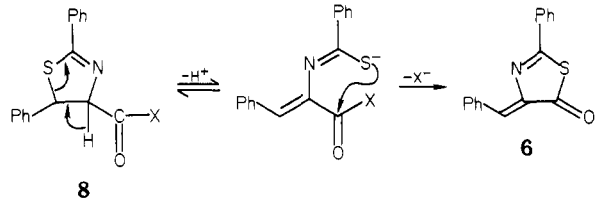
when 8 was warmed in acetic anhydride, the thiazolone 6 was regenerated. The mass spectrum of 8 gave no molecular ion, but the base peak appeared at m/e 116.9, corresponding to a phenylazirine fragment formed by loss of CO_2 and PhCHS.

Having the thiazolinecarboxylic acid 8 in hand, we allowed its conversion into the tripeptide 11 using the standard DCC/HOBt coupling procedure. Interestingly,



11

when 8 was converted into its *N*-hydroxysuccinimide ester for use in peptide synthesis, TLC showed that the ester was unstable and that the thiazolone 6 was the major product formed on standing. Apparently, even gentle



8 6

activation of the carboxyl function of 8 encourages loss of the C-4 proton with subsequent ring closure to the thiazolone, the last step being promoted by the presence of a good leaving group (X) on the carbonyl function.

The two peptides prepared were, in fact, moderate inhibitors of ACE: **2a**, IC_{50} 1.0×10^{-5} M and **11**, 7.6×10^{-6} M.

Experimental Section

All melting points were determined on a Thomas-Hoover capillary melting-point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer Model 297 infrared spectrophotometer with polystyrene as the standard. The ^1H NMR spectra were recorded on a Varian EM-390 spectrometer using tetramethylsilane (Me_4Si) as the internal standard. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter. Reagent grade DMF was treated with Dowex-50, distilled from CaO, and stored over 4-Å molecular sieves. Ethyl acetate was distilled from P_2O_5 and stored over 4-Å molecular sieves. THF was distilled from potassium metal and stored over molecular sieves. The diethyl ether and hexanes used in recrystallizations were stored over sodium ribbon. *N*-Methylmorpholine and triethylamine were distilled from and stored over CaO. All other reagents and solvents were used as received.

Thin-layer chromatography was performed on Whatman NK6F 1 × 3 precoated silica gel plates and TLC plates were visualized with UV light, 1% ninhydrin reagent, or 10% H_2SO_4 char. The following solvent systems were employed: (1) chloroform–methanol (1:1), (2) chloroform–methanol (10:1), (3) chloroform–methanol–acetic acid (25:5:1), (4) ether–petroleum ether (1:2), (5) ether–petroleum ether (1:1), (6) methanol–ethyl acetate (2:1), (7) chloroform–methanol (4:1), (8) 1-butanol–acetic acid–water (4:1:5) organic phase, (9) ethyl acetate–G mix (1:4) (G mix = pyridine–acetic acid–water, 20:6:11.), (10) 1-butanol–acetic acid–water–ethyl acetate (1:1:1:1), (11) 1-butanol–acetic acid–pyridine-water (4:1:1:2).

Gly-Pro-OH. To a solution of 3.68 g (12.0 mmol) Z-Gly-Pro-OH[13] in 80 mL of methanol was added 0.5 mL of glacial acetic acid and 300 mg of 10% Pd/C. Hydrogen was bubbled through the stirred suspension for 5 h. The catalyst was removed by filtration through Celite and the mother liquor concentrated and dried in vacuo to afford a colorless oil, which was redissolved in 50 mL of methanol. Diethyl ether was added until the solution was slightly turbid. A few drops of methanol were added. Crystallization occurred upon standing at room temperature. The mixture was chilled at 0 °C for 2 h, filtered, and dried in vacuo to provide 1.93 g (93%) of pure Gly-Pro; mp 205 °C dec; R_f[10] 0.67; ^1H NMR (CD_3CO_2D) δ 4.60 (m, 1 H, Pro C_α-H), 4.13 and 4.03 (2 s, 2 H, Gly C_α-H), 3.64 (m, 2 H, Pro C_δ-H), 2.43–1.73 (m, 4 H, Pro C_β-H and C_γ-H); IR (KBr disk) 3400–2300 ($^+NH_3$), 1680 (amide I), 1600 (CO_2^-), 1520 (amide II) cm^{-1}.

Anal. Calcd for $C_7H_{12}N_2O_3 \cdot 0.25H_2O$: C, 47.5; H, 7.13; N, 15.85. Found: C, 47.59; H, 7.18; N, 15.82.

***N*-Bz-Δ^Z-Phe-Gly-Pro-OH (2a).** To a solution of 1.25 g (5.00 mmol) of 3[9a] in 20 mL of dry DMF was added a suspension of 1.06 g (6.0 mmol) of Gly-Pro-OH and 1.05 mL (6.0 mmol) of diisopropylethylamine in 10 mL of dry DMF. After 24 h of stirring, at room temperature, the DMF was removed in vacuo and the residue obtained was partitioned between 25 mL of 10% Na_2CO_3 and 25 mL of diethyl ether. The aqueous layer was extracted twice with 25-mL portions of ether, chilled to 0 °C, and acidified to pH 2 (pH paper) with saturated $KHSO_4$. The solid was fiiltered, washed with cold water, and recrystallized from 50% aqeuous ethanol. The solid obtained was dried in a drying pistol at 40 °C (refluxing CH_2Cl_2) and 0.1 mmHg in the presence of P_2O_5 to afford 1.38 g (65%) of **2a** as fine white needles: mp 155.5–156.5 °C; R_f[3] 0.66; $[\alpha]^{26}_D$ −52.4° (*c* 1.02, methanol); ^1H NMR (CD_3OD) δ 8.14–7.93 (m, 2 H, ortho protons of NBz group), 7.73–7.26 (m, 9 H, Ar H and vinyl protons), 4.43 (dd, 1 H, Pro C_α-H, J = 5 Hz), 4.17 and 3.94 (2 d, 2 H, Gly C_α-H, J = 7, and 5 Hz, respectively), 3.62 (t[br], 2 H, Pro C_δ-H, J = 4 Hz), 2.34–1.81 (m, 4 H, Pro C_β-H and C_γ-H); IR (KBr disk) 3600–2200 (NH and CO_2H), 1735 (acid carbonyl), 1655 and 1620 (amide I), 1510 and 1460 (amide II) cm^{-1}.

Anal. Calcd for $C_{23}H_{23}N_3O_5 \cdot H_2O$: C, 62.85; H, 5.73; N, 9.56. Found: C, 62.83; H, 5.73; N, 9.55.

2,5-Diphenyl-2-thiazoline-4-carboxylic Acid (8). A suspension of 2.66 g (10.0 mmol) of 6[10] and 30 mL of 1 N NaOH was refluxed for 2 h. The bright-yellow solution was allowed to cool to room temperature, extracted twice with 15-mL portions of diethyl ether, acidified to pH 1 (pH paper) with concentrated HCl, and extracted with diethyl ether (3 × 25 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo to give a light-yellow solid. Two recrystallizations from benzene provided 1.68 g (59%) of 8: mp 138–140 °C dec (lit.[10] 136–137 °C); R_f[3] 0.62; ^1H NMR ($CDCl_3$, Me_2SO-d_6) δ 8.10–7.90 (m, 2 H, ortho protons of 2-phenyl group), 7.58–7.20 (m, 8 H, Ar H), 5.50 and 5.43 (2 H, 2 d, **CHCH**); IR (KBr disk) 3400–2100 (CO_2), 1740 (acid carbonyl), 1590 (C=N) cm^{-1}.

Anal. Calcd for $C_{16}H_{13}NO_3S$: C, 67.82; H, 4.62; N, 4.94; S, 11.32. Found: C, 67.86; H, 4.66; N, 4.92; S, 11.30.

Conversion of 8 into 3. A suspension of 100 mg (0.35 mmol) of 8 and 3 mL of 1 N HCl was refluxed overnight. After cooling to room temperature, the aqueous solution was diluted to 10 mL with water and extracted with three 5-mL portions of diethyl ether, concentrated, and dried in vacuo to provide 65 mg (79%) of crude 9. The major product gave positive color test for ninhydrin, chlorine–tolidine reagent, and sodium nitroprusside–NH_3 reagent and afforded the following TLC data: R_f[11] 0.63, R_f[9] 0.58, R_f[12] 0.67. This product was dissolved in 2 mL of dry pyridine and 0.4 mL of benzoyl chloride was added. After 4 h, the reaction mixture was diluted with 5 mL of 5% $NaHCO_3$ and stirred for 2 h. The mixture was extracted with ethyl acetate (3 × 25 mL), and the combined organic extracts were washed with 5% NaCl (3 × 10 mL), dried ($MgSO_4$), and concentrated in vacuo. Recrystallization

(12) Cook, A. H.; Harris, G.; Heilbron, S. I. *J. Chem. Soc.* **1948**, 1060. The rearrangement of 2-(ethylthio)-4-benzylidene-5(4*H*)-thiazolone into 5-phenyl-2-thiazolidone-4-carboxylic acid in hot NaOH is described.

(13) Anderson, G. W.; Zimmerman, J. E.; Callahan, F. M. *J. Am. Chem. Soc.* **1964**, *86*, 1839.

from benzene–hexanes provided 48 mg (69%) of **3**: mp 161–162 °C. The ^{1}H NMR and IR spectra of this product were identical with those of **3**.

Methyl *trans*-2,5-Diphenyl-2-thiazoline-5-carboxylate (10). To a solution of 100 mg (0.35 mmol) of **8** in 10 mL of diethyl ether was added dropwise an 0.3 N ethereal solution of diazomethane until the evolution of N_2 ceased. A few drops of glacial acetic acid was added. The solution was extracted with 5% $NaHCO_3$ (3 × 10 mL) and saturated NaCl (2 × 5 mL), dried ($MgSO_4$), and concentrated in vacuo. The residue obtained was purified by preparative TLC on a Whatman MK6F 20 × 10 cm plate, employing diethyl ether/hexanes (1:2) as eluant to provide 100 mg (88%) of **10** as a yellow oil: R_f[4] 0.66; ^{1}H NMR ($CDCl_3$) δ 8.10–7.84 (m, 2 H, ortho protons of 2-phenyl group), 7.60–7.18 (m, 8 H, Ar H), 5.42 and 5.31 (2 d, 2 H, CHCH), 3.76 (s, 3 H, CH_3); IR (NaCl, neat), 1743 (ester carbonyl), 1600 (C=N) cm^{-1}.

***N*-(*trans*-2,4-Diphenyl-2-thiazolin-5-ylcarbonyl)glycyl-proline (11).** To a chilled (0 °C) solution of 0.28 g (1.0 mmol) of **8** in 6 mL of dry THF was added 0.15 g (1.0 mmol) of HOBt and 0.23 g (1.1 mmol) of DCCI. The mixture was stirred for 0.5 h at 0 °C and 0.5 h at room temperature and filtered, and a solution of 0.27 g (1.5 mmol) of Gly-Pro-OH and 0.16 g (1.5 mmol) of Na_2CO_3 in 20 mL of water was added. The reaction mixture was stirred overnight. The THF was removed in vacuo, and the aqueous solution was extracted twice with 25-mL portions of diethyl ether, acidified to pH 2 (pH paper) with saturated $KHSO_4$, and extracted with chloroform (3 × 25 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo to provide a light-yellow solid. Recrystallization from ethyl acetate provided 120 mg of **11**. The solid was dissolved in 1 mL of methanol and the solution applied to a 2.5 × 90 cm column of Sephadex LH-20, employing methanol as eluant at a flow rate of 5 mL/h. Fractions 70–85 were combined and concentrated in vacuo to dryness and the residue obtained was recrystallized from ethyl acetate to provide 82 mg (19%) of pure **11**: mp 173–174 °C dec, R_f[3] 0.67; $[\alpha]^{26}_D$ −19.2° (*c* 1.04, methanol); ^{1}H NMR ($CDCl_3$) δ 8.90 (s, 1 H, NH), 8.13–7.82 (m, 2 H, ortho protons of 2-phenyl group), 7.70 (m, 1 H, NH), 7.62–7.20 (m, 8 H, Ar H), 5.58 and 5.40 (2 d, 2 H, CHCH, J = 5 Hz), 4.54 (m, 1 H, Pro C_α-H), 4.10 (m, 2 H, Gly C_α-H), 3.53 (m, 2 H, Pro C_δ-H), 2.06 (m, 4 H, Pro C_β-H and C_γ-H); IR (KBr disk) 3350 (NH), 3600–2600 (CO_2H), 1740 (acid carbonyl), 1620 (amide I), 1600 (C=N), 1520 (amide II) cm^{-1}.

Anal. Calcd for $C_{23}H_{23}N_3O_4S$: C, 63.14; H, 5.30; N, 9.60; S, 7.33. Found: C, 63.06; H, 5.33; N, 9.59; S, 7.28.

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Registry No. L-(*Z*)-**2a**, 82865-27-8; (*Z*)-**3**, 17606-70-1; L-(*Z*)-**4**, 82865-28-9; (*Z*)-**6**, 82865-29-0; *trans*-**8**, 82865-30-3; **9**, 4371-55-5; *trans*-**10**, 82865-31-4; L-**11**, 82865-32-5; ACE, 9015-82-1; Gly-L-Pro-OH, 704-15-4; Gly-L-Pro-OMe, 53309-02-7; *Z*-Gly-L-Pro-OH, 1160-54-9; benzoyl chloride, 98-88-4.

Differentiation of Brominated Biphenyls by Carbon-13 Nuclear Magnetic Resonance and Gas Chromatography/Mass Spectrometry

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The chemical structures of specific brominated biphenyls were determined unambiguously from their ^{1}H NMR, ^{13}C NMR, and mass spectra. With these model compounds and the previously characterized components of a technical mixture, the existence of an ortho effect in the mass spectral fragmentation of brominated biphenyls was established. Comparisons of the relative abundances of the $[M - Br]^+$ fragment, the $[M - 2Br]^{+\cdot}$ fragment, and the molecular ion $[M]^{+\cdot}$ distinguish brominated biphenyls with 2,2′, 2,2′,6, or 2,2′,6,6′ substitution from biphenyls without ortho,ortho′ substitution and from biphenyls with 2,2′,3 substitution. The order of brominated biphenyl elution from nonpolar GC columns correlates with retention index measurements obtained for analogous chlorinated biphenyls. Use of the ortho effect combined with the order of GC elution appears to be a reliable method for the partial structural differentiation of brominated biphenyl isomers by standard GC/MS techniques.

Polybrominated biphenyls (PBBs) are of current interest because of their contribution to several instances of widespread environmental contamination, their established animal toxicity, their presence in human tissues, and thus their possible human health effects.[1-5] Investigations centering about PBB identification, partitioning in tissues, and biological effects have been numerous.[6-15] The metabolism and toxicity of PBBs vary with isomer structure.[16,17] Understanding the health effects of PBBs, therefore, clearly requires knowledge of the characteristics of specific brominated biphenyls.

Characterization of brominated biphenyls is complicated by the fact that there are 209 possible PBB congeners, excluding optical isomers that result from hindered rota-

(1) Dicarlo, F. J.; Seifter, J.; DeCarlo, V. J. *Environ. Health Perspect.* **1978**, *23*, 351.
(2) Carter, L. J. *Science* **1976**, *192*, 240.
(3) Kay, K. *Environ. Res.* **1977**, *13*, 74.
(4) DHEW: Final Report of the Subcommittee on the Health Effects of Polychlorinated Biphenyls and Polybrominated Biphenyls of the DHEW Committee to Coordinate Toxicology and Related Programs. *Environ. Health Perspect.* **1978**, *24*, 129.
(5) Bekesi, J. G.; Holland, J. F.; Fishbein, A. S.; Rom, W.; Wolff, M. S.; Selikoff, I. J. *Science* **1978**, *199*, 1207.
(6) Andersson, K.; Norström, A.; Rappe, C.; Rasmuson, B.; Swahlin, H. *Environ. Qual. Saf. Suppl.* 1975 **1978**, *3*, 798.
(7) Sundström, G.; Hutzinger, O.; Safe, S. *Chemosphere* **1976**, *1*, 11.
(8) Norström, A.; Andersson, K.; Rappe, C. *Chemosphere* **1976**, *1*, 255.
(9) Jacobs, L. W.; Chou, S.; Tiedje, J. M. *J. Agric. Food Chem.* **1976**, *24*, 1198.
(10) De Kok, J. J.; De Kok, A.; Brinkman, U. A. Th.; Kok, R. M. *J. Chromatogr.* **1977**, *142*, 367.
(11) Hass, J. R.; McConnell, E. E.; Harvan, D. J. *J. Agric. Food Chem.* **1978**, *26*, 94.
(12) Moore, R. W.; O'Connor, J. V.; Aust, S. D. *Bull. Environ. Contam. Toxicol.* **1978**, *20*, 478.
(13) Dannan, G. A.; Moore, R. W.; Aust, S. D. *Environ. Health Perspect.* **1978**, *23*, 51.
(14) Moore, R. W.; Dannan, G. A.; Aust, S. D. *Pharmacologist* **1978**, *20*, 251.
(15) Moore, R. W.; Aust, S. D. *Biochem. Biophys. Res. Commun.* **1978**, *84*, 936.
(16) Domino, E. F.; Fivenson, D. P.; Domino, S. E. *Drug Metab. Dispos.* **1980**, *8*, 332.
(17) Moore, R. W.; Sleight, S. D.; Aust, S. D. *Toxicol. Appl. Pharmacol.* **1978**, *44*, 309.