(48) Yields are substantially lower and the product much less pure if the $LiAlH_4$ used in this reaction is not of high quality; cf. P. G. Schroeder, M.S. Thesis, University of Arkansas, 1968.
(49) G. I. Poos, G. E. Arth, R. E. Beyler, and L. H. Sarett, *J. Am. Chem. Soc.*, **75**, 427 (1953).
(50) This procedure was developed by Dr. E. J. Schindler of these laboratories.
(51) We thank the Eastman Chemical Co. for a generous sample of this compound.
(52) Several commercial samples of isovaleric acid were contaminated with an unidentified impurity which we were unable to remove. Consequently the isovaleric acid for this work was prepared by carbonation of the isobutyl Grignard reagent by adapting the general procedure of H. Gilman and R. H. Kirby in "Organic Syntheses", Collect. Vol. I, Wiley, New York, N.Y., 1932, p 361. It was converted to its acid chloride by the method of H. C. Brown, *J. Am. Chem. Soc.*, **60**, 1325 (1938).
(53) This procedure was developed by Drs. D. C. Shew and C. W. Sigel of these laboratories.

Structures of Some *Knightia deplanchei* Alkaloids[1]

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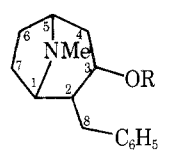
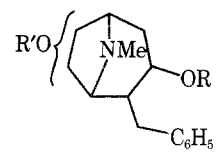
^{13}C NMR spectroscopy is utilized for the determination of the stereostructures of five alkaloids of the 2-benzyltropine type. Intramolecular hydrogen bonding by 6β- or 7β-hydroxy groups is shown to alter the normal conformation of the tropane *N*-methyl group. This phenomenon is modified greatly in a protic medium.

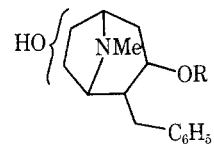
Several tropane alkaloids have been isolated recently from the New Caledonian plant *Knightia deplanchei* Vieill. ex Brongn. et Gris. Two compounds were shown to be 2-benzyltropanol derivatives **1a** and **1b** by spectral analyses[2,3] and synthesis,[4] while three others, **2a, 3a,** and **4**, were described as oxygenated variants of their congeners.[2,3] A ^{13}C NMR spectral study now has been undertaken in order to determine the complete structures of the last three substances and the stereochemistry of all five tropane bases.



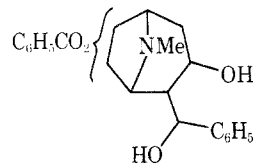
1a, R = COC_6H_5
b, R = Ac
c, R = H



2a, R = H; R′ = COC_6H_5
b, R = R′ = COC_6H_5
c, R = R′ = H

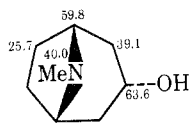


3a, R = *trans*-$COCH{=}CHC_6H_5$
b, R = H



4

A previous investigation has revealed the carbon shifts of structurally simple tropanes, tropine (**5**), pseudotropine (**6**), and their benzoates (**7** and **8**, respectively) (δ values depicted on the formulas), and related alkaloids.[5] While the shift data could serve the present study well, their value was obscured in part by a recent report by Simeral and Maciel in which the assignment of the C(2), C(4) shifts of tropine (**5**) had been allotted to C(6), C(7).[7] Since, further, the tropine (**5**) spectrum had been taken in water solution, while the earlier δ values were obtained on deuteriochloroform solutions, a shift reevaluation had to precede the alkaloid structure study.



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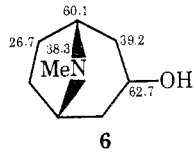

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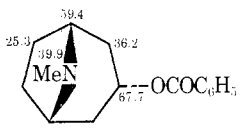

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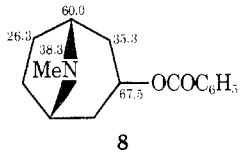


8

The coupling characteristics of the two carbon pairs were utilized to determine unambiguously the proper shift assignment. A series of off-resonance decoupling experiments designed to optimize possible carbon–hydrogen virtual coupling of C(6) were performed on deuteriochloroform solutions of tropine (**5**) and pseudotropine (**6**). The combination of strong residual coupling between C(6) and H(6α), but weak C(6)–H(7α) interaction and strong H(6α)–H(7α) coupling due to the identity of the two hydrogen shifts was expected to lead to virtual coupling of this ABX system.[8,9] The strong dissimilarity of the H(2) shifts from the resonances of the vicinal hydrogens precludes any C(2) virtual coupling. The observation of second-order coupling in the C(6) signal showed the earlier shift assignment[5] for compounds **5**–**8** to be correct. Furthermore, a similar observation in off-resonance decoupled spectra of an aqueous tropine (**5**) solution indicated the need for the reversal of the recent shift designation of C(2), C(4) and C(6), C(7).[7,10]

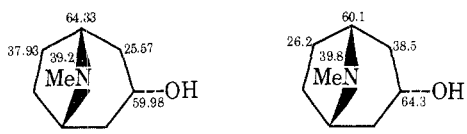
Close inspection of the fully proton-decoupled ^{13}C NMR spectrum of an aqueous solution of tropine (**5**) showed all lines except the oxymethine signal to be broad, the *N*-methyl and C(2), C(4) centers revealing the largest linewidths. While the broad 60.1-ppm signal was less intense than the 64.3-ppm resonance, the former covered an area nearly twice as large, showing it to constitute a two-carbon signal.[11] As a consequence the Simeral and Maciel assignment of the former resonance to C(3) and the latter to C(1), C(5)[7] needs reversal. The new assignment was confirmed by the residual coupling characteristics of the two resonances. A single-frequency, off-resonance decoupled (sford) spectrum in which the decoupling frequency was localized at the upfield end of the ^1H NMR spectrum exhibited expectedly lower residual coupling of the aminometh-

Table I
Chemical Shifts[a]

	1a	1b[b]	1c	2a	2b	2c	3a[c]	3b	4
C(1)	63.2	63.3	63.8	64.2	63.5	62.8	70.7	71.6	62.5
C(2)	45.8	45.4	46.3	44.9	44.3	40.0	38.6	40.1	49.4
C(3)	69.7	69.3	65.6	65.4	69.3	66.0	69.3	66.1	64.6
C(4)	37.0	37.0	39.9	36.9	34.0	32.7	31.0	34.4	36.0
C(5)	59.6	59.8	60.0	66.5	65.8	67.4	58.3	58.8	66.3
C(6)	25.3	25.3	25.3	80.5	80.0	76.2	40.9	40.8	80.3
C(7)	21.9	21.6	21.8	32.2	32.7	37.1	72.8	73.3	33.1
C(8)	35.2	35.1	35.4	35.2	35.1	35.1	35.0	35.1	74.2
i-C	139.0	139.2	140.1	140.0	138.7	139.8	138.9	139.8	142.7
o-C	127.9	128.1	127.9	128.1	128.1	128.3	127.9	128.3	126.4
m-C	128.7	128.7	128.7	128.8	128.7	128.7	128.7	128.7	128.4
p-C	125.7	125.8	125.5	125.7	126.0	125.8	126.0	125.8	127.8
NMe	40.3	40.4	40.3	40.6	40.6	36.8	36.7	37.1	40.3

[a] The δ values are in parts per million downfield from Me_4Si; $\delta(Me_4Si) = \delta(CDCl_3) + 76.9$ ppm. [b] The methyl and carbonyl shifts of the acetyl group are 21.3 and 169.9 ppm, respectively. [c] The cinnamyl group possesses the following shifts: C=O 165.6, α-C 118.0, β-C 144.8, *ipso*-C 134.0, *o*-C 128.7, *m*-C 128.3, and *p*-C 130.2 ppm.

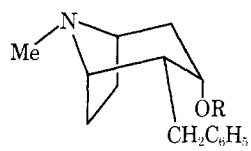
ine doublet than the oxymethine doublet.[8] Thus the former carbon shifts for tropine (**5**) in water illustrated on formula **9**[7] have been revised to the δ values portrayed on formula **10**.[12]



9 **10**

With the earlier chemical shift assignment of tropine (**5**)[5] and hence of pseudotropine (**6**) and their benzoates (**7** and **8**, respectively) reaffirmed, the ^{13}C NMR spectral analysis of the *Knightia deplanchei* could be undertaken. The introduction of a benzyl group at C(2) of either of the benzoates of tropine (**7**) or pseudotropine (**8**) can be expected to cause deshielding of all nonaromatic methines except C(5). This fact and the strong dissimilarity of the substituents at C(1), C(2), and C(3) permits the shift designation of all methines of alkaloid **1a**. The differentiation of the methylenes of the latter is based on the recognition of the benzylic methylene from its residual coupling characteristics (vide supra) and of C(6) and C(7) through their virtual coupling behavior (vide supra) as well as on C(7) being shielded by the proximate benzyl group. The benzoate carbons of compounds **1a, 2a, 2b,** and **4** exhibit the following signals: C=O 165.8 ± 0.4 ppm, *ipso*-C 130.3 ± 0.2 ppm, *o*-C 129.2 ± 0.1 ppm, *m*-C 128.1 ± 0.1 ppm, and *p*-C 132.7 ± 0.2 ppm. Both alkaloids **1a** and **1b** have been shown to yield the same hydrolysis product (**1c**).[2] The ^{13}C NMR analysis of **1b** and **1c** follow arguments similar to those for **1a** above. The chemical shifts of all compounds **1** are listed in Table I.

The C(6) and *N*-methyl resonances are diagnostic of the C(3) stereochemistry of tropine benzoate (**7**) and its C(3) epimer **8**. Similarly, the C(3) configuration of the two esters is reflected in the dissimilar C(3) as well as C(4) shift difference between esters **7** and **8** and alcohols **5** and **6**, respectively. Thus the C(3) and C(4) $\Delta\delta$ (**7**–**5**) are 4.1 and −2.9 ppm, respectively, and the corresponding $\Delta\delta$(**8**–**6**) values are 4.8 and −3.9 ppm, respectively. On this basis alkaloid **1a** possesses the C(3) stereochemistry of tropine benzoate (**7**) and the chair conformation of its piperidine ring. The close similarity of the C(4) shift of **1a** with that of **7** and the appreciable shielding of C(7) by the benzyl group indicate the latter to be equatorially oriented and hence cis to the C(3) substituent. As a consequence compounds **1a, 1b,** and **1c** possess stereostructures **11a, 11b,** and **11c**, respectively.

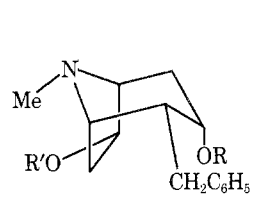


11a, R = COC_6H_5
b, R = Ac
c, R = H

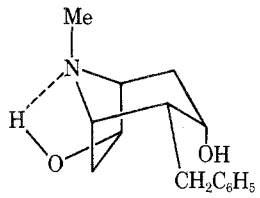
Inspection of the ^{13}C NMR spectra of alkaloid **2a** and its derivatives **2b** and **2c** reveals the effect of the substitution of a methylene group by an oxymethine function in the tropine nucleus of **1**. Comparison of the field positions and residual couplings (vide supra) of the methines of **2a** distinguishes its oxymethines from the aminomethines and C(2). In view of the close similarity of one of the oxymethine shifts with the C(3) shift of **1c** the 80.5 ppm signal can be allotted to the new oxymethine. Furthermore, the closeness of one of the aminomethine shifts to that of C(1) of **1c** and the strongly lower field position of the remaining aminomethine permits differentiation between the two methines and placement of the benzoyloxy group at C(6) of the tropine nucleus. In contrast to the behavior of compounds **1** the alkaloid **2a** reveals in its sford spectrum a methylene group with grossly magnetically nonequivalent hydrogens which as a consequence of the distant, new oxymethine cannot be the benzylic methylene function. Recognition of the latter is based on its larger residual coupling (vide supra) among the two remaining methylene signals. Carbons 4 and 7 can be distinguished from each other by the C(4) shift perturbation encountered on benzoylation of the 3-hydroxy group (cf. **2b**).

In view of the δ values of C(3) and C(8) being nearly identical in **2a** and **1c** as well as in **2b** and **1a** the stereochemistry of C(2) and C(3) must be the same in compounds **2** as in substances **1**. The C(6) configuration is based on an interpretation of the dramatic shift alteration in glycol **2c**, the product of hydrolysis of alkaloid **2a**. The ^{13}C NMR spectra of **2c** show strong shielding of C(2), C(4), and the *N*-methyl group, characteristic of reciprocal γ effects. Since the *N*-methyl inversion, leading to 1,3-diaxial interactions with H(2β) and H(4β), can be justified only on the basis of hydrogen bonding between the nitrogen electron pair and the 6-hydroxy group, the latter must possess an exo orientation. Thus **2a** and **2b** are represented by stereostructures **12a** and **12b**, respectively, while **2c** can be depicted by **13**. The chemical shifts of all three substances are listed in Table I.[13]

Previous work has shown that the hydrolysis product

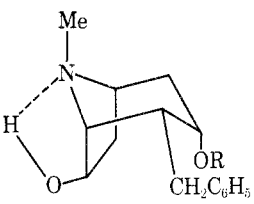


12a, R = H; R′ = COC_6H_5
b, R = R′ = COC_6H_5

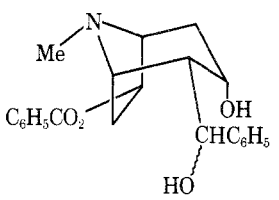


13

(**3b**) of alkaloid **3a** is an isomer of glycol **2c**.[2] The ^{13}C NMR spectra of **3a** and **3b** verify this fact and from the high-field position of the signals of the *N*-methyl group, C(2) and C(4) reveal the extra hydroxy group to occupy a 7β site of the tropine skeleton. In contrast to the behavior of **2c**, glycol **3b** feels a β effect at C(1) from the adjacent hydroxy group and reveals the presence of nonequivalent hydrogens at C(6). The fact of the C(3) and C(8) shifts being nearly identical in glycols **2c** and **3b** proves the identity of the C(2) and C(3) configurations in alkaloid **3a** with those of the natural bases **1a**, **1b**, and **2a**.[15] Thus substances **3a** and **3b** are represented fully by formulas **14a** and **14b**, respectively. Their chemical shifts are listed in Table I.



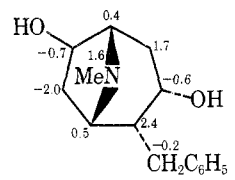
14a, R = $COCH{=}CHC_6H_5$-*t*
b, R = H



15

The determination of the location of the benzoyloxy unit of alkaloid **4** is based on consideration of the aminomethine shifts. The C(1) resonance is expected to be upfield that of compounds **1c**, **2a**, and **2b** owing to the γ effect exerted by the 8-hydroxy group in the case of the benzoyloxy moiety being located at C(6) and upfield of the C(1) shift of **3a** and **3b** in the event of the pyrrolidino substituent being held at C(7). Carbon 5 can be expected to be unaffected by the 8-hydroxy group and therefore to reflect the placement of the benzoyloxy function directly through its δ value. These arguments lead to the deposition of the benzoyloxy group at C(6) and the nearly identical C(5) and C(6) shifts of **4** and **2a** suggest an exo configuration for the substituent. The *N*-methyl shift of alkaloid **4** shows the nitrogen substituent to be equatorially disposed to the piperidine ring and supports the presence of a tropine (**5**), rather than pseudotropine (**6**) structure. Finally, the only slight perturbation of the C(4) shift of **4**, compared with **2a**, indicates the hydroxybenzyl group to possess an equatorial conformation. As a consequence the stereostructure of **4** is **15**. Its carbon shifts are depicted in Table I.

As the conformational behavior of compounds **2c** (**13**), **3a** (**14a**), and **3b** (**14b**) reveals, the introduction of 6β- or 7β-hydroxy groups into the tropane skeleton produces intramolecular hydrogen bonding forcing the *N*-methyl group into an axial orientation with respect to the piperidine ring. This phenomenon would be expected to be modified in protic media in which competition with intermolecular hydrogen bonding could take place. This change can be observed through the ^{13}C NMR spectra of several of the above compounds in 25% methanol–deuteriochloroform solution. While alcohols **1c** and **2a** show only minimal shift changes of up to 0.5 ppm, dramatic alterations occur in compounds **2c**, **3a**, and **3b**. The $\Delta\delta$ values [δ(MeOH–$CDCl_3$) – $\delta(CDCl_3)$], depicted on **16**, **17**, and **18**, respectively, exhibit deshielding of C(2) and C(4) in all three substances and simultaneous shielding of C(7) in **2c** and C(6) in **3a** and **3b**, characteristic of the *N*-methyl group assuming an increasing equatorial conformation with respect to the piperidine ring.



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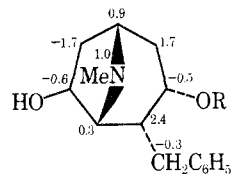
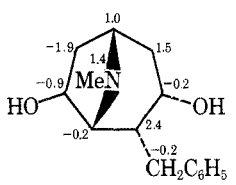


17, R = $COCH{=}CHC_6H_5$-*t*



18

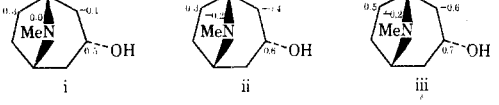
Experimental Section

All carbon shifts were recorded on a Varian XL-100-15 NMR spectrometer operating at 25.20 MHz in the Fourier transform mode. The shifts on formulas **10** and iii are referenced to internal dioxane [$\delta(Me_4Si)$ = δ(dioxane) + 67.4 ppm]. The shift differences denoted on formulas **16**, **17**, **18**, i, ii, and v are with reference to internal methanol [$\delta(Me_4Si)$ = δ(MeOH) + 49.5 ppm].

Registry No.—**1a**, 50656-86-5; **1b**, 50656-87-6; **1c**, 56816-03-6; **2a**, 50656-88-7; **2b**, 56761-52-5; **2c**, 56761-53-6; **3a**, 56761-54-7; **3b**, 56761-55-8; **4**, 55249-52-0.

References and Notes

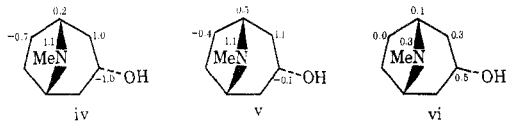
(1) Carbon-13 Nuclear Magnetic Resonance Spectroscopy of Naturally Occurring Substances. XXXVI. For the preceding paper see B. L. Buckwalter, I. R. Burfitt, A. A. Nagel, E. Wenkert, and F. Näf, *Helv. Chim. Acta*, **58**, 1567 (1975).
(2) C. Kan-Fan and M. Lounasmaa, *Acta Chem. Scand.*, **27**, 1039 (j973).
(3) M. Lounasmaa, *Planta Medica*, **27**, 83 (1975).
(4) M. Lounasmaa and C.-J. Johansson, *Tetrahedron Lett.*, 2509 (1974).
(5) E. Wenkert, J. S. Bindra, C.-J. Chang, D. W. Cochran, and F. M. Schell, *Acc. Chem. Res.*, **7**, 46 (1974).[6]
(6) In the description of the carbon shifts of pseudotropine (**6**) those of the *N*-methyl group and of C(2), C(4) had been mixed up inadvertently.
(7) L. Simeral and G. E. Maciel, *Org. Magn. Reson.*, **6**, 226 (1974).
(8) E. Wenkert, B. L. Buckwalter, I. R. Burfitt, M. J. Gašić, H. E. Gottlieb, E. W. Hagaman, F. M. Schell, and P. M. Wovkulich, "Carbon-13 Nuclear Magnetic Resonance Spectroscopy of Naturally Occurring Substances", in G. C. Levy, "Topics in Carbon-13 NMR Spectroscopy", Vol. II, Wiley, New York, N.Y., in press.
(9) The same argument applies to the ABX system of C(6), H(6β), and H(7β).
(10) The shift reversal probably applies also to the δ values presented for an aqueous solution of atropine.[7]
(11) Whereas in Fourier transform spectroscopy the signal area loses much of its diagnostic value for carbon counting, it is still applicable in those cases in which the carbon sites to be compared are substituted by an equal number of hydrogens and thus, to a first approximation, the carbon relaxation times are similar.
(12) Tropine (**5**) shows unusual shift perturbations in a variable solvent, temperature, and concentration study. While at 29° the base shows line broadening in water solution, it retains sharp lines in deuteriochloroform, methanol, and 20% water–methanol solutions. The $\Delta\delta$ values [δ(solvent) – $\delta(CDCl_3)$] depicted on i, ii, and iii indicate the shift variations at



i ii iii

different carbon sites in methanol, water–methanol, and water solutions, respectively. Lowering of the temperature causes all signals except the oxymethine resonance to broaden and finally to resharpen at new field positions. Contrastingly, the ^1H NMR spectrum in deuteriochloroform solution remains nearly unaffected through the same temperature range. The temperature of greatest linewidth of the carbon signals is −15 to −23° in deuteriochloroform and 0 to −11° in 20% water–

methanol solutions. The $\Delta\delta$ values [$\delta(-30°) - \delta(29°)$] for the two solutions are portrayed on formulas iv and v, respectively. A threefold dilu-



iv v vi

tion of a 2 *M* deuteriochloroform solution which had yielded the shifts denoted on formula **5** leads to the $\Delta\delta$ values [δ(dil) − δ(concd)] illustrated on formula vi.

(13) Curiously, the introduction of the benzoyloxy group at C(6) shields C(2) and C(4), the latter twice as much as the former. Since the 6-oxy substituent is γ-equatorially oriented to C(4) within the cycloheptane nucleus, part of its shielding may be due to the effect recently noted in six-membered ring systems.[14]

(14) E. L. Eliel, W. F. Bailey, L. D. Kopp, R. L. Willer, D. M. Grant, R. Bertrand, K. A. Christensen, D. K. Dalling, W. M. Duch, E. Wenkert, F. M. Schell, and D. W. Cochran, *J. Am. Chem. Soc.*, **97**, 322 (1975).

(15) The shift alterations in the pyrrolidine ring on introduction of a 6β-hydroxy group (**1c** → **2c**) can be used to confirm the pyrrolidine carbon shifts in a 7β-hydroxy compound (**3b**). The $\Delta\delta$(**2c** − **1c**) values for C(5), C(6), C(7), and C(1) are 7.4, 50.9, 15.3, and −1.0 ppm, respectively. Their sequential application to C(1), C(7), C(6), and C(5) of **3b** yields the theoretical values of 71.2, 72.7, 40.6, and 59.0 ppm, respectively, in close agreement with the found shifts.

Sulfur-Containing Polypeptides. XVIII. Unambiguous Synthesis of the Parallel and Antiparallel Isomers of Some Bis-Cystine Peptides[1-3]

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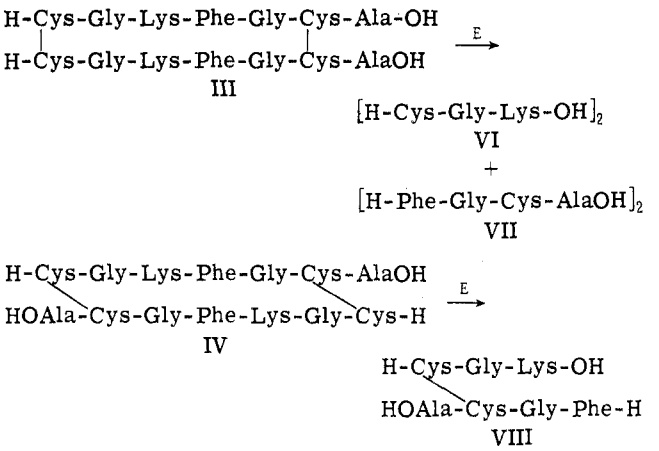
The synthesis of the parallel (III) and antiparallel (IV) isomers and cyclic monomer (V) of the L-cystylglycyl-L-lysyl-L-phenylalanylglycyl-L-cystyl-L-alanine system via the thiocyanogen–sulfenyl thiocyanate method is described. Enzymic degradation of III and IV using trypsin and α-chymotrypsin were employed to establish the pairing of the cystine residues. The thiocyanogen–sulfenyl thiocyanate method has been shown to proceed without intermediate disulfide interchange.

As part of a program directed toward the development of methods for the laboratory synthesis of polypeptides containing several cystine residues,[1] unequivocal methods for the stepwise and selective conversion of various S-protected cysteine thiols to cystine residues with the desired sulfur pairing have been studied. A route which indicated some promise[4] has been the utilization of *S*-trityl and *S*-benzhydryl thioethers of cysteine and subsequent selective oxidative removal of these protective groups by thiocyanogen or sulfenyl thiocyanates of cysteine, the former group being removed without catalysis, the latter requiring acidic conditions.

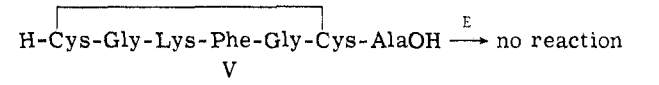
Unfortunately, the isomeric parallel (I) and antiparallel (II) bis-cystine dimers prepared by the sulfenyl thiocyanate method exhibited virtually identical physical properties and appeared to differ only in the magnitude of their optical rotations (the parallel dimer of the L-peptide having the greater negative rotation). Furthermore, the parallel and antiparallel dimers of a particular series could not be distinguished by thin layer or column chromatography.[4,5] In view of the possibility that the acid conditions[4] required for oxidative removal of the *S*-benzhydryl groups (or a thiol–disulfide interchange process as shown in Scheme I) could in fact lead to equilibration of the dimers, which could not be distinguished analytically, a bis-cystine system in which the purity of the isomeric dimers could be unequivocally established was developed.

The peptides of choice were the molecules III–V; treatment of III with trypsin should yield two cleavage products (VI, VII). Similar treatment of IV with trypsin would afford only VIII. Enzymic digestion with α-chymotrypsin should lead to a similar situation with cleavage occurring at the amide bond between Phe-Gly in both III and IV.

Relatively little is known of the parameters which effect enzymic cleavage of cystine containing peptides. Schally and Barrett[6] demonstrated that the antiparallel dimer of [Lys^8] vasopressin (40-membered ring) was cleaved at the



Phe-Gln bond by α-chymotrypsin. Walter and Hoffman[7] showed that oxytocin, lysine vasopressin, and arginine vasopressin (20-membered rings) were resistant to the action of α-chymotrypsin at an enzyme–substrate ratio of 1:300, whereas the corresponding S-alkylated nonapeptides were smoothly cleaved by the enzyme. The cyclic pentapeptide cyclo(Gly-Lys-Gly-Lys-Gly) is resistant to trypsin hydrolysis (15-membered ring) although the linear system is cleaved.[8] The cyclic monomer V (20-membered ring) was therefore expected to be inert to either the action of trypsin or α-chymotrypsin. If these expectations were realized III,



IV, and V could be distinguished from one another (since cyclic monomers have different TLC mobilities than bis dimers) and the specificity of the thiocyanogen–sulfenyl thiocyanate reaction could be established.

The heptapeptide derivatives required for the synthesis