Facile Carbon–Sulfur Bond Cleavage Leading to an Enethiolate. Formation of a Dipeptide Containing Dehydrocysteine and Dehydrovaline[1]

NELSON J. LEONARD AND ROBERT Y. NING

Noyes Chemical Laboratory, University of Illinois, Urbana, Illinois

Received August 19, 1966

3D-Carbomethoxy-2,2-dimethyl-5-oxo-6-phenylacetamido-2,3,4,5-tetrahydro-1,4-thiazepine (I) undergoes a rapid elimination reaction with ring opening when treated with an equivalent amount of sodium hydroxide or sodium methoxide at room temperature to give the stable, isolable sodium salt of methyl N-phenylacetyl-α',β'-dehydrocysteinyl-α,β-dehydrovalinate (II), an enethiol. Compound II gave S-(2,4-dinitrophenyl) and S-benzyl derivatives readily with 1-chloro-2,4-dinitrobenzene and benzyl bromide, respectively. Acidification of an aqueous solution of salt II gave a polymer (V), which apparently formed from the thioaldehyde generated through the tautomerization of the enethiol. In contrast, the saturated analog of I, 3D-carbomethoxy-2,2-dimethyl-5-oxo-6D-phenylacetamidoperhydro-1,4-thiazepine (VI), undergoes a similar elimination reaction at a much slower rate.

Some derivatives of 1,4-thiazepines are of interest as possible synthetic and biosynthetic precursors in a transannular route to pencillins.[2] The synthesis and certain representative reactions of 3D-carbomethoxy-2,2-dimethyl-5-oxo-6-phenylacetamido-2,3,4,5-tetrahydro-1,4-thiazepine (I) and 3D-carbomethoxy-2,2-dimethyl-5-oxo-6D-phenylacetamidoperhydro-1,4-thiazepine (VI) have been reported previously.[3-6] When we attempted saponification of I under the mild conditions of 1 *N* sodium hydroxide in 80% aqueous methanol at room temperature, a very rapid elimination reaction occurred, involving removal of a proton from C_3 and cleavage of the C_2–S bond, to give quantitatively the sodium salt of methyl N-phenylacetyl-α',β'-dehydrocysteinyl-α,β-dehydrovalinate (II). The ease with which this reaction occurred was demonstrable in numerous ways. When an ethanolic solution of I [λ_{max} 305 mμ (ϵ 5260) and 235 mμ (ϵ 9650)] was passed through a column of quaternary ammonium hydroxide ion-exchange resin, the ultraviolet spectrum of the effluent showed a flat base line, indicating adsorption on the column as ammonium salt. Addition of 1 drop of 4 *N* aqueous sodium hydroxide solution to 3 ml of an ethanolic sample solution of I in a spectrophotometer cell shifted the maxima at 305 and 235 mμ to the more intense maxima of II at 336 mμ (ϵ 10,500) and 253 mμ (ϵ 13,000) almost instantaneously. In contrast, addition of 1 drop of triethylamine caused no change for at least 10 min. The loss of the optical center at C_3 suffered by I in the conversion was demonstrated when the specific rotation of I, measured in ethanol, rose from -105 to 0° as soon as an equivalent amount of sodium methoxide was added. The sodium salt II itself could be crystallized, in the absence of water and alcohols, from ethyl acetate; the isolated yield of light yellow grains, obtained by treatment of I with an exact equivalent of sodium methoxide, was 84%, mp 168–170°, with the correct analysis for $C_{17}H_{19}N_2NaO_4S$. In addition to the ultraviolet spectrum, the infrared and nmr spectra were consistent with the assigned structure II. This enethiolate was characterized further by preparations of derivatives through alkylations with 1-chloro-2,4-dinitrobenzene and benzyl bromide. In each case, monoalkylation occurred on the anionic sulfur, and high yields of the S-(2,4-dinitrophenyl) and S-benzyl derivatives, III and IV, mp 181–183° and 154–155°, respectively, were obtained. Both derivatives gave ultraviolet, infrared, and nmr spectra, as well as elemental analyses, in agreement with the assigned structures (see Scheme I).

It is interesting to note that salt II is the stabilized form of the tautomeric thioaldehyde, the free acid (IX) of which has been implicated[7-9] as a possible biosynthetic precursor of penicillin (see Scheme II) proposed as an alternative to the transannular route involving 1,4-thiazepines.

Thioaldehydes are very unstable as monomeric species; they readily form cyclic trimers or higher polymers.[10-14] For this reason, monomers have rarely been characterized. In like manner, neither the free enethiol corresponding to II nor the tautomeric thioaldehyde was isolable. A gummy complex mixture was obtained by acidification of the alkaline, aqueous solution of the salt II to pH 3.8, followed by extractions with chloroform. Separation of this mixture by column chromatography gave a fraction, representing 32% yield, of amorphous polythioaldehyde, melting indefinitely in the range 135–163°. This material had the correct analysis for the monomeric composition $C_{17}H_{20}N_2O_4S$, with an average molecular weight, determined osmometrically in benzene, of 3600 ± 200, which corresponds to an average chain length of about ten monomeric units (mol wt 348). Indications of the fact that the acrylic double bond in the dehydrovaline portion of the molecule survived the polymerization were found in the infrared and nmr spectra of the isolated polymer. The carbonyl stretching frequency of the methyl ester remained at a relatively low value of 1720 cm^{-1}, and the singlet signals for the *gem*-dimethyl protons appeared at τ values of 8.37 and 7.98

(1) This work was supported in part by a research grant (USPHS-GM-05829) from the National Institutes of Health, U. S. Public Health Service, to whom we are pleased to acknowledge our thanks.

(2) All cogent references are cited in footnotes 3 and 4.

(3) N. J. Leonard and G. E. Wilson, Jr., *J. Am. Chem. Soc.*, **86**, 5307 (1964).

(4) N. J. Leonard and R. Y. Ning, *J. Org. Chem.*, **31**, 3928 (1966).

(5) N. J. Leonard and G. E. Wilson, Jr., *Tetrahedron Letters*, 1465 (1964).

(6) N. J. Leonard and G. E. Wilson, Jr., *ibid.*, 1471 (1964).

(7) H. R. V. Arnstein and J. C. Crawhall, *Biochem. J.*, **67**, 180 (1957).

(8) E. P. Abraham and G. G. F. Newton, "Biochemistry of Antibiotics," Proceedings of the Fourth International Congress of Biochemistry, Vienna, Vol. V, Pergamon Press Inc., New York, N. Y., 1958, p 54.

(9) D. J. D. Hockenhull in "Biochemistry of Industrial Micro-organisms," C. Rainbow and A. H. Rose, Ed., Academic Press Inc., New York, N. Y., 1963, pp 244–246.

(10) E. Campaigne, *Chem. Rev.*, **39**, 1 (1946).

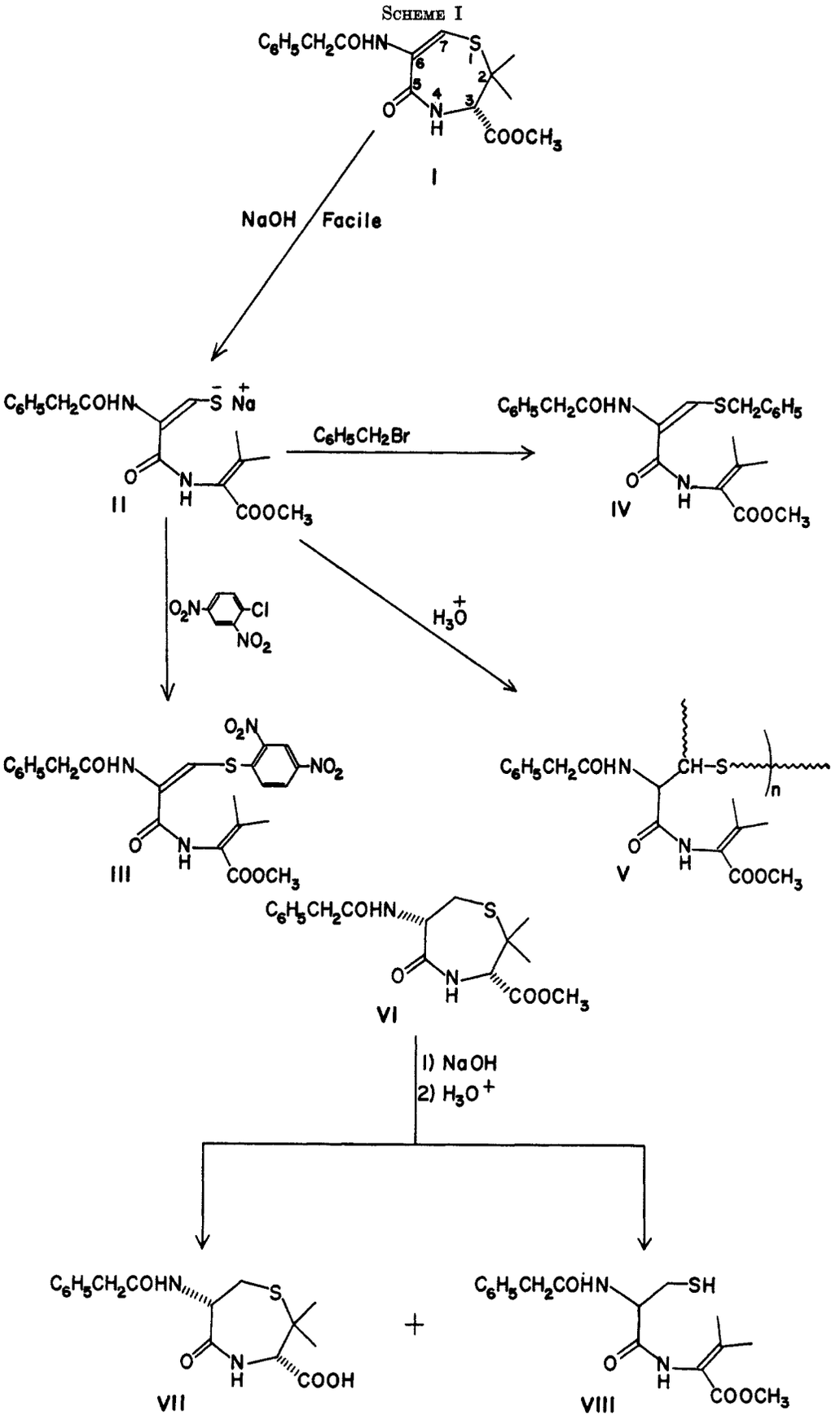
(11) E. Campaigne in "The Chemistry of the Carbonyl Group," S. Patai, Ed., Interscience Publishers, Inc., New York, N. Y., 1966, Chapter 17.

(12) A. Schönberg and M. Z. Barakat, *J. Chem. Soc.*, 693 (1947).

(13) J. A. Stanfield and L. B. Reynolds, Jr., *J. Am. Chem. Soc.*, **74**, 2878 (1952).

(14) N. J. Leonard and W. K. Musker, *ibid.*, **81**, 5631 (1959).

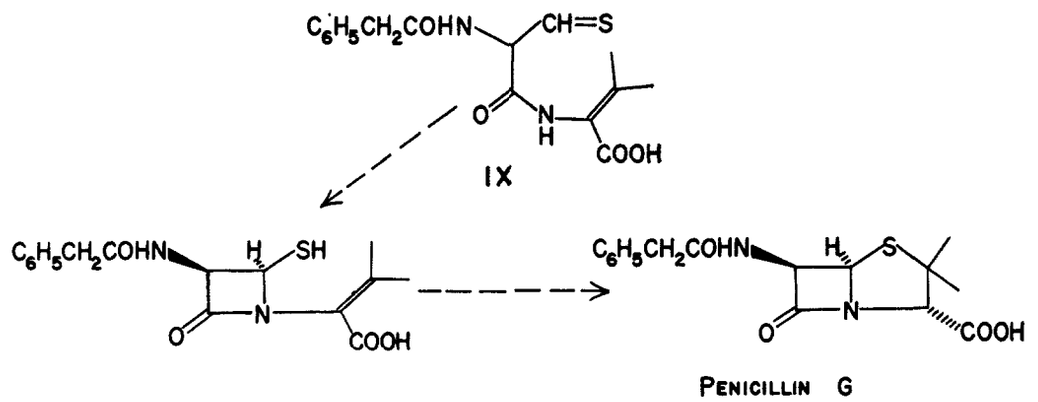
SCHEME I



ppm. It is worth noting that the infrared spectrum of the total mixture before separation closely resembled the spectrum of the isolated fraction. It may be inferred from this that the bulk of the less readily purified material was composed of similar polymers of smaller or larger molecular weight.

A study of the saponification of 3D-carbomethoxy-2,2-dimethyl-5-oxo-6D-phenylacetamidoperhydro-1,4-thiazepine (VI) under mild alkaline conditions provided an interesting comparison with I in their tendencies to undergo elimination. Treatment of VI with 1 *N* sodium hydroxide in 80% aqueous methanol at 24°

SCHEME II

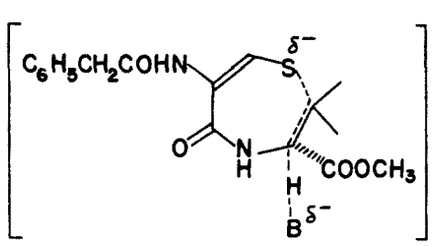


PENICILLIN G

for 1 hr converted it completely to water-soluble salts. Acidification of an aqueous solution to pH 1 precipitated a mixture of methyl N-phenylacetyl-D-cysteinyl-α,β-dehydrovalinate (VIII) and 3D-carboxy-2,2-dimethyl-5-oxo-6D-phenylacetamidoperhydro-1,4-thiazepine (VII). The thiol VIII (20%) was separated from the carboxylic acid VII (36% isolated) by selective solution of the latter from the mixture with aqueous sodium bicarbonate. Compound VIII gave a positive sodium nitroprusside spot test for thiols. After recrystallizations from methanol–water, it melted at 198–200°. Elemental analysis and nmr and infrared spectra were consistent with the assigned structure. The acid VII was readily identified by direct comparison of melting point and nmr and infrared spectra with those of the known compound.[4]

To the extent that sodium hydroxide did cause elimination in VI, the predominance of removal of a proton from C_3 accompanied by the cleavage of the C_2–S bond over possible proton removal from C_6 and cleavage of the C_7–S bond is noteworthy. This is probably due in part to greater activation by the carbomethoxyl for C_3 hydrogen removal, toward carbanion formation, than by the carboxamide group for C_6 hydrogen removal and in part to the greater relief of steric strain accompanying C_2–S cleavage than C_7–S cleavage.

We have noted the pronounced increase in the ease of elimination when a double bond was introduced α to the sulfur, as in 3D-carbomethoxy-2,2-dimethyl-5-oxo-6-phenylacetamido-2,3,4,5-tetrahydro-1,4-thiazepine (I). An interesting direct comparison was made when half-equivalent amounts of sodium hydroxide were added to separate, equal volumes of 50% aqueous ethanolic solutions containing equal amounts of I and VI and the drop in pH of the solutions was monitored with glass electrodes over a period of 30 min (see Figure 1). The pH of the solution containing I dropped from 11.1 to 9.0 in the first 5 min while that of the solution containing VI dropped only 0.2 unit in the same period.



X

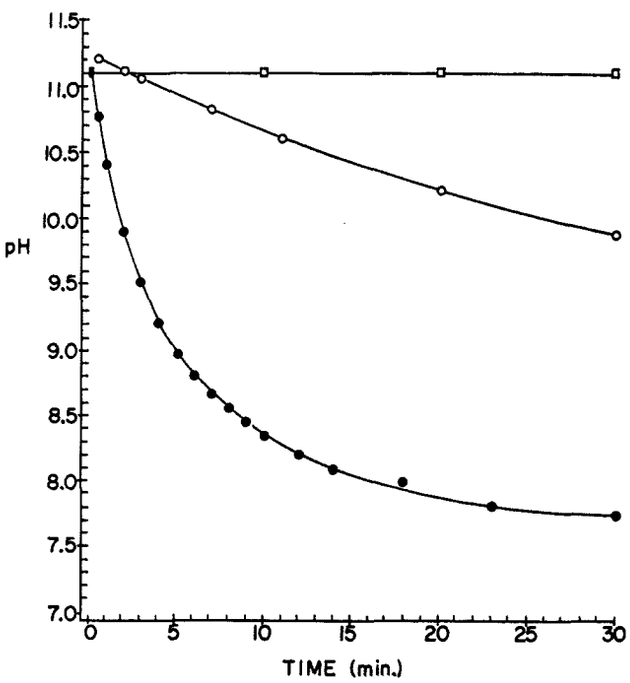


Figure 1.—Relative sensitivity of 0.0286 mmole of each compound to 0.014 mmole of NaOH in 10 ml of 50% aqueous ethanol at 25 ± 2°: □, blank; ○, 3D-carbomethoxy-2,2-dimethyl-5-oxo-6D-phenylacetamidoperhydro-1,4-thiazepine (VI); and ●, 3D-carbomethoxy-2,2-dimethyl-5-oxo-6-phenylacetamido-2,3,4,5-tetrahydro-1,4-thiazepine (I).

It is likely that the double bond plays a vital role in the resonance stabilization of the developing sulfur anion, and a possible transition state of the base-catalyzed reaction is pictured in X. A transition state for the elimination in VI may be pictured in the same manner. The degree to which the presence of the double bond stabilizes the developing anion is hard to estimate, although it is clear that the negative charge can be readily distributed to C_6 and to the C_5 oxo group. The delocalization of charge in the developing sulfur anion, with the breaking of the C_2–S bond in I, is sufficient to account for the difference in relative rates of elimination between I and VI, as judged by the difference in pK_a values between thiophenol and alkyl mercaptan.[15–17] In addition, however, it can be seen from molecular models that the un-

(15) H. C. Brown, D. H. McDaniel, and O. Häfliger in "Determination of Organic Structures by Physical Methods," E. A. Braude and F. C. Nachod, Ed., Academic Press Inc., New York, N. Y., 1955, Chapter 14.
(16) W. H. Fletcher, *J. Am. Chem. Soc.*, **68**, 2726 (1946).
(17) G. Schwarzenbach and E. Rudin, *Helv. Chim. Acta*, **22**, 360 (1939).

saturated compound I can readily assume a conformation permitting *trans* coplanarity of C_3–H and C_2–S, whereas the saturated compound VI can conform to analogous *trans* geometry only by overcoming considerable steric repulsion.

Finally, it should be mentioned that other enethiols have been isolated and alkylated.[11,18-23] Compound II appears to be the first enethiolate isolated which is derived from a thioaldehyde.

Experimental Section[24]

3D-Carbomethoxy-2,2-dimethyl-5-oxo-6D-phenylacetamidoperhydro-1,4-thiazepine (VI).—Compound VI was prepared following the procedure of Leonard and Wilson.[3] It may also be synthesized *via* the alternative route of Leonard and Ning.[4]

3D-Carbomethoxy-2,2-dimethyl-5-oxo-6-phenylacetamido-2,3,4,5-tetrahydro-1,4-thiazepine (I).—Compound I was prepared, following the procedure of Leonard and Wilson,[3] from the synthesis, followed by chlorination, of 3D-carbomethoxy-2,2-dimethyl-5-oxo-6D-phenylacetamidoperhydro-1,4-thiazepine (VI). To optimize yields and to avoid the inconvenience of having to separate unreacted starting material and the oxidative by-product of I, methyl α-D-isopropenyl-4-phenylacetamido-3-isothiazolone-2-acetate, from the desired product, it was found that the use of exactly 1 molar equiv of chlorine was important. The stock solution of chlorine in carbon tetrachloride was standardized just prior to use. Measured amounts of the chlorine solution were added to an aqueous solution containing excess potassium iodide, and the iodine liberated was titrated with standard thiosulfate solution using starch as indicator. Compound I, as obtained after purification by column chromatography on silica gel, melted at 139–140°, yield 38%.

Some Demonstrations of Extreme Lability of 3D-Carbomethoxy-2,2-dimethyl-5-oxo-6-phenylacetamido-2,3,4,5-tetrahydro-1,4-thiazepine (I) toward Hydroxyl and Methoxyl Ions.[25] A.—Addition of 1 drop of 4 *N* aqueous sodium hydroxide to a 3-ml sample solution of I in a spectrophotometer cell brought about an instantaneous quantitative change in the ultraviolet spectrum consisting of maxima at 235 mμ (ε 9650) and 305 mμ (ε 5260) to that of the sodium salt of methyl N-phenylacetyl-α′,β′-dehydrocysteinyl-α,β-dehydrovalinate (II) with maxima at 253 mμ (ε 13,100) and 335 mμ (ε 10,600). This spectrum of the alkaline solution was unchanged for many hours.

B.—The specfic rotation of I ($[\alpha]^{25}D$ −105°, 2% in ethanol) rose instantaneously to 0 when an equivalent amount of sodium methoxide was added.

C.—A column of quaternary ammonium hydroxide ion-exchange resin was prepared by packing 2 g of Dowex 1-8X resin in an 8-mm column followed by thorough washing with methanol. Sufficient I was dissolved in 20 ml of ethanol to give an ultraviolet absorbance of 0.9 at 253 mμ. After a single passage through the column, the spectrum of the solution showed a flat base line.

D.—A solution of 58.8 mg (0.669 mmole) of I in 30 ml of 50% aqueous ethanol was "titrated" with 0.094 *N* aqueous sodium hydroxide. Each addition of base was followed by a period of stirring at room temperature, varying between 3 and 20 min, until the pH change monitored by glass electrodes became negligible. A curve plotted for pH against milliliters of base added was found to be similar to that of acid–base titration, with an inflection occurring at a volume of sodium hydroxide added 7% short of theoretical equivalence point for 1 mole of I to 1 mole of base.

Sodium Salt of Methyl N-Phenylacetyl-α′,β′-dehydrocysteinyl-α,β-dehydrovalinate (II).—A solution of 30 mg (0.55 mmole) of sodium methoxide[26] in a small amount of anhydrous methanol was added at room temperature to a methanolic solution containing 200 mg (0.575 mmole) of 3D-carbomethoxy-2,2-dimethyl-5-oxo-6-phenylacetamido-2,3,4,5-tetrahydro-1,4-thiazepine (I), so that the total volume was about 6 ml. After 5 min, the solution was evaporated to dryness. The residual solid was dissolved in 6 ml of ethyl acetate, followed by addition of 2 ml of benzene and evaporation to dryness. Last traces of solvent were removed by pumping at room temperature under 0.05 mm. The dry solid was redissolved in a minimum amount of ethyl acetate without heating. After standing at 5° for 1.5 days, a yellow mother liquor was withdrawn, and a layer of granular, light yellow crystals was washed twice by decantation with ethyl acetate at room temperature, then pumped under vacuum to dryness. These crysals were found to be analytically pure without recrystallization: yield, 170 mg (84%); mp 168–170° dec (darkening from 165°); nmr τ values (15% in DMSO-d_6) 8.13 (6 H, broad singlet, *gem*-dimethyl) 6.49 (2 H, singlet, CH_2), 6.40 (3 H, singlet, OCH_3), 2.71 (5 H, singlet, phenyl), 1.60 (overlapping singlets, 2 H, one of which was exchangeable with D_2O, NH, CH), and −3.02 (1 H, singlet, NH); nmr spectrum in D_2O (15%, containing a trace of solid NaOH) gave singlets at −0.78 (CH), 0.00 (phenyl), +3.60 (OCH_3), +3.68 (CH_2), +5.32 and +5.42 ppm (*gem*-dimethyl) (chemical shift values from the phenyl peak used as reference); ν_{max}^{Nujol} 3270, 1705, 1642, 1580, 1535, 1350, 1340, 1225, 980, 890, and 765 cm^{-1}; λ_{max}^{EtOH} 336 mμ (ε 10,500) and 253 mμ (ε 13,000). The compound was optically inactive and the crystals were nonhygroscopic.

Anal. Calcd for $C_{17}H_{19}N_2NaO_4S$: C, 55.13; H, 5.17; N, 7.56; S, 8.66. Found: C, 55.33; H, 5.14; N, 7.30; S, 8.46.

The presence of sodium was confirmed by the residue of sodium salts upon ignition of sample in oxygen.

Methyl N-Phenylacetyl-S-(2,4-dinitrophenyl)dehydrocysteinyl-α,β-dehydrovalinate (III).—Sodium methoxide (15.6 mg, 0.288 mmole) dissolved in a little ethanol was added to 100 mg (0.288 mmole) of 3D-carbomethoxy-2,2-dimethyl-5-oxo-6-phenylacetamido-2,3,4,5-tetrahydro-1,4-thiazepine (I) dissolved in 5 ml of ethanol. The mixed solutions were allowed to stand for 0.5 hr at room temperature; then 58.5 mg (0.288 mmole) of 1-chloro-2,4-dinitrobenzene dissolved in 2 ml ethanol was added. After stirring at room temperature for 5 min, 6 drops of water was added, and golden yellow needles of III formed instantly. The mixture was allowed to stand at −15° for 2 hr; then the needles were harvested and washed with cold ethanol followed by ether: yield 120 mg (81%); mp 181–183° (mp 192–194° after recrystallizations from benzene–cyclohexane); nmr τ values (15% in DMSO-d_6) 8.17 (3 H, singlet, CH_3), 7.98 (3 H, singlet, CH_3), 6.42 (3 H, singlet, OCH_3), 6.23 (2 H, singlet, CH_2), 2.67 (5 H, singlet, phenyl), and 2.88 to −0.56 (6 H, a series of small peaks); ν_{max}^{Nujol} 3395, 3270, 1720, 1665, 1640, 1597, 1528, 1350, 1220, 862, and 735 cm^{-1}; λ_{max}^{EtOH} 368 mμ (ε 12,300), 280 mμ (sh) (ε 11,400).

Anal. Calcd for $C_{23}H_{22}N_4O_8S$: C, 53.70; H, 4.31; N, 10.89; S, 6.22. Found: C, 53.46; H, 4.23; N, 10.60; S, 6.36.

Methyl N-Phenylacetyl-S-benzyldehydrocysteinyl-α,β-dehydrovalinate (IV).—A solution of 1.00 g (2.88 mmoles) of 3D-carbomethoxy-2,2-dimethyl-5-oxo-6-phenylacetamido-2,3,4,5-tetrahydro-1,4-thiazepine (I) in 15 ml of methanol was mixed with 0.156 g (2.88 mmoles) of sodium methoxide dissolved in 10 ml of methanol. After standing at room temperature for 20 min, a solution of 0.513 g (3.00 mmoles) of benzyl bromide dissolved in 25 ml of methanol was added. After occasional stirring at room temperature for 0.5 hr, the mixture was evaporated to dryness. Sodium bromide was separated from the residual solids by treatment with 40 ml of chloroform. Filtration and evapora-

(18) S. K. Mitra, *J. Indian Chem. Soc.*, **10**, 71 (1933).

(19) Z. Reyes and R. M. Silverstein, *J. Am. Chem. Soc.*, **80**, 6367, 6373 (1958).

(20) E. Campaigne and B. E. Edwards, *J. Org. Chem.*, **27**, 3760 (1962).

(21) S. H. H. Chaston, S. E. Livingstone, T. N. Lockyer, V. A. Pickles, and J. S. Shannon, *Australian J. Chem.*, **18**, 673 (1965).

(22) S. Bleisch and R. Mayer, *Z. Chem.*, **4**, 146 (1964).

(23) E. Campaigne and R. E. Cline, *J. Org. Chem.*, **21**, 32 (1956).

(24) All melting points are corrected. The nmr spectra were obtained with a Varian Associates Model A-60 spectrometer. Unless otherwise stated, the chemical-shift values were measured using tetramethylsilane as an internal standard (τ = 10 ppm). Whenever practicable, the signals owing to active hydrogens, such as NH and OH, were identified through the loss of these signals after 2 drops of deuterium oxide had been added to the nmr sample tube, the mixture was shaken for 5 min or more, and the spectra were measured. Infrared spectra were obtained with a Perkin-Elmer automatic recording infrared spectrophotometer Model 521. Ultraviolet spectra were obtained using a Cary Model 15 spectrophotometer. Optical rotations were measured on a Bendix Ericsson ETL-NPL automatic polarimeter, Type 143 A, in a 1-cm cell using a sodium lamp. Unless otherwise noted, evaporation of all solvents was conducted under vacuum using a Büchi rotary evaporator at a bath temperature of less than 40°. All solvents used were of reagent grade purity. The authors wish to thank Mr. Joseph Nemeth and his associates for microanalyses and osmometric molecular weight determinations.

(25) Triethylamine has no effect on I.

(26) A batch obtained from Matheson Coleman and Bell gave an equivalent weight of 55 upon titration in water with standard hydrochloric acid and could be easily and accurately weighed.

tion of the chloroform solution followed by recrystallizations of the residue from benzene gave 1.16 g (92%) of colorless rods: mp 154–155°; λ_{max}^{EtOH} 299 mμ (ε 15,700); nmr τ values (15% in $CDCl_3$) 8.25 (3 H, singlet, CH_3), 7.89 (3 H, singlet, CH_3), 6.41 (2 H, singlet, $C_6H_5CH_2$), 6.34 (3 H, singlet, OCH_3), 6.02 (2 H, singlet, SCH_2), 2.73 (10 H, singlet, two phenyls), 2.03 (1 H, broad singlet, CH), and 1.85 (2 H, broad, two NH); ν_{max}^{Nujol} 3300, 3225, 1714, 1662, 1635, 1512, 1325, 1215, 892, 855, 798, 770, and 702 cm^{-1}.

Anal. Calcd for $C_{24}H_{26}N_2O_4S$: C, 65.74; H, 5.98; N, 6.39; S, 7.30. Found: C, 65.89; H, 5.88; N, 6.19; S, 7.56.

Tests of Acidities of the Amide Hydrogens of Methyl N-Phenylacetyl-S-benzyldehydrocysteinyl-α,β-dehydrovalinate (IV) and the Tendencies to Form Conjugated Anionic Species. A.—To a solution of 12.5 mg (0.0286 mmole) of IV in 5 ml of ethanol was added 5 ml water followed immediately by 0.15 ml of 0.094 *N* NaOH (0.014 mmole). The pH of the solution was monitored, while stirring at room temperature, with glass electrodes over a period of 30 min. The initial pH of 11.4 dropped only 0.2 unit during this time. It was concluded that the pK_a values of the active hydrogens were greater than 11.4.

B.—Two drops each of triethylamine, 4 *N* aqueous NaOH solution, and 5% sodium methoxide in ethanol were added separately to 4 ml of ethanolic sample solutions of IV in ultraviolet spectrophotometer cells, and changes in the spectra were recorded. Triethylamine had no effect, while both sodium hydroxide and sodium methoxide shifted the band at λ_{max} 299 mμ to λ_{max} 303 mμ, accompanied by a slight decrease in intensity. Using 1,2-dimethoxyethane which had been dried over lithium aluminum hydride as the solvent, in the same cells, addition of 10 mg of sodium hydride, followed by shaking, shifted the curve from λ_{max} 300 mμ to λ_{max} 311 mμ, accompanied by a slight increase in intensity.

Acidolysis of the Sodium Salt of Methyl N-Phenylacetyl-α′,β′-dehydrocysteinyl-α,β-dehydrovalinate (II) in Aqueous Solution.—To a solution of 1.05 g (3.0 mmoles) of 3D-carbomethoxy-2,2-dimethyl-5-oxo-6-phenylacetamido-2,3,4,5-tetrahydro-1,4-thiazepine (I) in 15 ml of methanol was added 5.0 ml of 2.5 *N* (12.5 mmoles) aqueous sodium hydroxide. After swirling the solution for 5 min at room temperature it became faintly yellow and was added slowly, with stirring, over a period of 3 min, to 50 ml of pH 4.6, 0.1 *M* monopotassium phosphate buffer solution. The resulting pH was 11.4. This alkaline solution gave only a trace of unidentifiable material upon repeated extractions with chloroform. About 13 ml of 1 *N* aqueous hydrochloric acid, which volume was determined by prior titration of stock solutions to be an exact equivalent of the sodium hydroxide used, was added with stirring from a buret to the aqueous solution. A copious, flocculent precipitate formed. The resulting pH of the solution was 3.8. The mixture was extracted with three volumes of chloroform. The combined chloroform extracts, after drying over anhydrous sodium sulfate and evaporation to dryness at 30°, gave 1.05 g of a colorless, glassy residue. Column chromatography of this material on 125 g of 100–200 mesh silica gel, using 2.5 l. of ethyl acetate, 1.5 l. of acetone, and 1 l. of 10% methanol in acetone as eluting solvents in this order, failed to separate the mixture into any discrete, crystallizable fractions. All the fractions eluted from the column by ethyl acetate and acetone were applied, using chloroform as solvent, to a second column of 50 g of neutral Woelm–Eschwege activity 1 alumina, packed in benzene, measuring 3 cm in diameter. Elution with a total of 300 ml of benzene and 300 ml of chloroform gave intractable fractions. Elution with 300 ml of 10% methanol in chloroform, however, gave fractions from which crystallizations from benzene–cyclohexane produced 340 mg (32%) of polythioaldehyde in an amorphous form, mp 120–160°. After two recrystallizations from acetone–cyclohexane followed by two recrystallizations from benzene–cyclohexane, 240 mg of the material was obtained: mp 135–163°; ν_{max}^{Nujol} 3260, 1721, 1642, 1496, 1305, 1225, and 1090 cm^{-1}; nmr τ values ($CDCl_3$) 8.37 (3 H, broad singlet, CH_3), 7.98 (3 H, broad singlet, CH_3), 6.45 (5 H, broad singlet, overlapping OCH_3 and benzyl CH_2), and 2.82 (5 H, singlet, phenyl); weak diffused signals in the τ = 5 ppm region were not recognizable. Osmometric molecular weight determination, using benzene as the solvent, gave a value of 3600 ± 200 which corresponds to an average composition of about 10 monomeric units (mol wt 348).

Saponification of 3D-Carbomethoxy-2,2-dimethyl-5-oxo-6D-phenylacetamidoperhydro-1,4-thiazepine (VI).—Addition of 350 mg (1.00 mmole) of VI to 4 ml of 1 *N* sodium hydroxide in 80% aqueous methanol gave a clear solution in 5 min. After standing for 1 hr at 24°, 15 ml of water was added. Extraction with two-portions of 20 ml of chloroform yielded only a trace of unidentifiable material. The alkaline, aqueous layer was acidified to pH 1, then extracted twice with chloroform, to give, after drying over anhydrous sodium sulfate and evaporation, 290 mg of a colorless, noncrystalline, solid material. Addition of 5% aqueous sodium bicarbonate dissolved the bulk of the solids, leaving 70 mg (20%) of crude, amorphous methyl N-phenylacetyl-D-cysteinyl-α,β-dehydrovalinate (VIII), mp 189–196°. After two recrystallizations by dissolving in minimum amounts of methanol at room temperature, then precipitation by addition of water, needles were obtained: mp 198–200°; ν_{max}^{Nujol} 3250, 1720, 1640, 1535, 1308, 1237, 1092, and 698 cm^{-1}; nmr τ values (CF_3COOH) 8.08 (3 H, singlet, CH_3), 7.75 (3 H, singlet, CH_3), 7.2–6.8 (2 H, multiplets, SCH_2), 6.13 (5 H, singlet, overlapping OCH_3 and benzyl CH_2), 5.25–4.85 (1 H, multiplet, CH), and 2.65 (5H, singlet, phenyl).

Anal. Calcd for $C_{17}H_{22}N_2O_4S$: C, 58.27; H, 6.33; N, 8.00; S, 9.13. Found: C, 58.51; H, 6.37; N, 7.88; S, 8.87.

The aqueous bicarbonate solution upon careful acidification to pH 1 and standing overnight at 5° gave 120 mg (36%) of 3D-carboxy-2,2-dimethyl-5-oxo-6D-phenylacetamidoperhydro-1,4-thiazepine (VII), identified by melting point and infrared and nmr spectra.[4]

A Direct Comparison of the Sensitivity of 3D-Carbomethoxy-2,2-dimethyl-5-oxo-6D-phenylacetamidoperhydro-1,4-thiazepine (VI) and 3D-Carbomethoxy-2,2-dimethyl-5-oxo-6-phenylacetamido-2,3,4,5-tetrahydro-1,4-thiazepine (I) toward Sodium Hydroxide in 50% Aqueous Ethanol.—Solutions of 10.0 mg (0.0286 and 0.0287 mmole, respectively) of VI and I in 5 ml of ethanol were diluted with equal volumes of water. To each solution was then added, in one portion, 0.5 equiv of sodium hydroxide (0.15 ml of 0.094 *N* NaOH), and the pH change was monitored, while stirring at room temperature, with glass electrodes. Values of pH taken over a period of 30 min at 25 ± 2° were plotted against time (Figure 1).

Registry No.—I, 903-21-9; II, 10028-08-7; III, 10028-09-8; IV, 10028-10-1; VIII, 10028-11-2.