Table IV. Characteristic Absorptions of S-Alkyl Analogs of Lincomycin Hydrochloride[a]

S substituent	Frequency, cps (*J*, cps) S-C*H*	C-C*H*$_3$	Anomeric H
Methyl	128	...	322 (5.5)
Ethyl	158 (7.5)	75	327 (5.5)
Isopropyl	178 (7.0)	78	331 (5.5)

[a] D_2O was the solvent.

for *cis* or *trans* configuration, and a methyl triplet at 68 and 72 cps, respectively.

When the R_3 substituent was an alkyl group other than methyl, the spectrum showed minor changes. The absence of the S-methyl singlet and appearance of the appropriate S-alkyl absorptions were noted, together with a small shift in the absorption frequency of the anomeric hydrogen (see Table IV).

When the 7-hydroxyl was replaced by a chlorine atom XV, profound changes were observed in the carbinol region of the spectrum (see Table II). It is important to note that the intramolecular hydrogen bond is also destroyed and the $J_{5,6}$ and $J_{6,7}$ coupling constants indicate a side-chain conformation similar to VIa.

Experimental Section

Nmr spectra were observed on a Varian A-60 spectrometer using solutions (*ca.* 0.4 ml, 0.3 *M*) of the samples in chloroform-*d* or deuterium oxide. Spectra were calibrated with internal tetramethylsilane (TMS) or sodium 2,2-dimethyl-2-silapentane-5-sulfonate (SDSS).[9] Spectra are calibrated in cps at 60 Mc to allow discussion of portions of multiplets.[28] Spin-decoupling experiments were performed with a Varian HR-100 spectrometer using a V-3521 integrator for field-sweep decoupling.[12]

The preparation of degradation products, derivatives, and analogs used in this study is described elsewhere.[3,5-7,21,22,26]

(28) G. Slomp, *J. Am. Chem. Soc.*, **84**, 673 (1962).

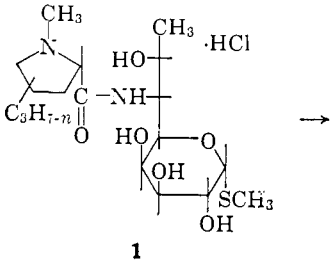
Lincomycin. V. Amino Acid Fragment[1]

Barney J. Magerlein, Robert D. Birkenmeyer, Ross R. Herr, and Fred Kagan

Contribution from the Research Laboratories of the Upjohn Company, Kalamazoo, Michigan. *Received December 20, 1966*

Abstract: The amino acid derived from the cleavage of the antibiotic lincomycin is shown to be *trans*-1-methyl-4-*n*-propyl-L-proline. A partial synthesis of lincomycin is described.

Cleavage of the antibiotic lincomycin hydrochloride (**1**) into methyl thiolincosaminide (**2**) and 1-methyl-*n*-propylproline (**3**) was described in previous papers of this series.[2,3] The determination of the position of the *n*-propyl substituent and the configuration at carbon atoms C_2 and C_4 in the proline fragment is now reported.



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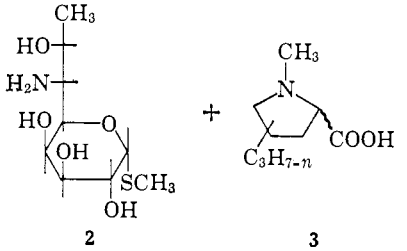


2 **3**

The over-all plan was to establish the position of the alkyl group and confirm the absolute stereochemistry of C-2 by synthesis of 3-, 4-, and 5-*n*-propyl-1-methyl-L-proline[4a] and then to determine the absolute configuration of C_4 by degradation.

The synthesis of 1-carbobenzoxy-4-methylene-L-proline (**5**) resulting from the action of methylenetriphenylphosphorane on 1-carbobenzoxy-4-keto-L-proline (**4**) was recently described.[4b] Under similar conditions *n*-propylidenetriphenylphosphorane failed to give the desired 4-propylidene compound **6**. However, a modification of the recently described sodium methylsulfinylcarbanion–dimethyl sulfoxide procedure[5] afforded **6** in 55% yield.

Although the double bond of **6** could be hydrogenated over a platinum catalyst to give **7**, concomitant hydrogenation and hydrogenolysis of **6** to yield **8** was generally more convenient. Reductive methylation of **8** afforded the methylated amino acid **9** in high yield. The saturated amino acids **7**, **8**, and **9** were obtained as *cis–trans* isomers, but separation of these isomers could not be achieved through chromatography, electrophoresis, or fractional crystallization.

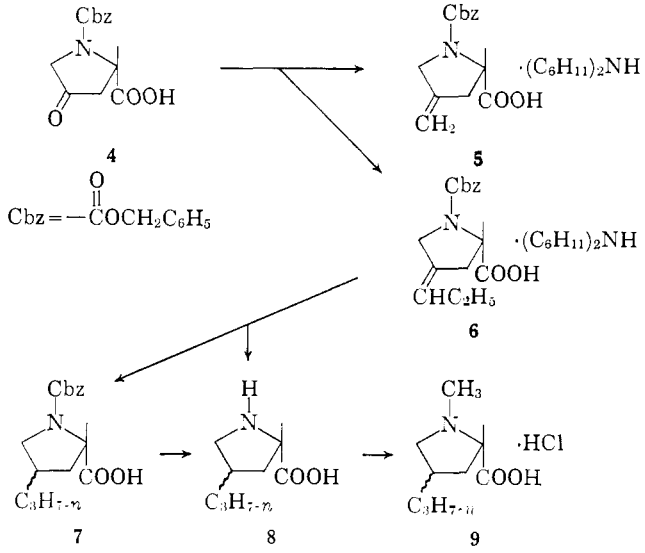
(1) Presented at the 148th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1964; Abstracts of Papers, p 6P. For a preliminary report *cf.* H. Hoeksema, B. Bannister, R. D. Birkenmeyer, F. Kagan, B. J. Magerlein, F. A. MacKellar, W. Schroeder, G. Slomp, and R. R. Herr, *J. Am. Chem. Soc.*, **86**, 4223 (1964).

(2) R. R. Herr and G. Slomp, *J. Am. Chem. Soc.*, **89**, 2444 (1967).

(3) W. P. Schroeder, B. Bannister, and H. Hoeksema, *ibid.*, **89**, 2448 (1967).

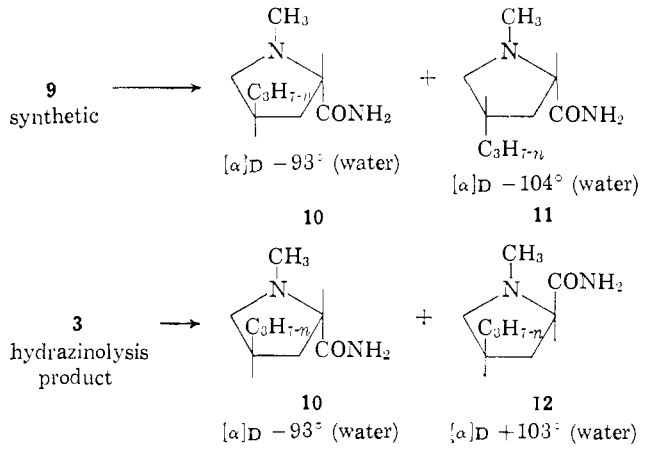
(4) (a) Rotational data suggested the L configuration for the carboxyl group. See ref 2. (b) M. Bethell, G. W. Kenner, and R. C. Sheppard, *Nature*, **194**, 864 (1962). The authors are indebted to Professor G. W. Kenner for experimental details of this reaction prior to publication.

(5) R. Greenwald, M. Chaykovsky, and E. J. Corey, *J. Org. Chem.*, **28**, 1128 (1963).



Conversion of the acids **9** to the amides afforded a mixture of *cis* and *trans* isomers **10** and **11** which was readily separated by chromatography over silica gel. One of these compounds was identical with the amide prepared from naturally occurring propylhygric acid, thus establishing the position of the propyl group at C-4 and the absolute configuration of the C-2 carbon atom as L. The absolute configuration of C-4 was established by degradation.

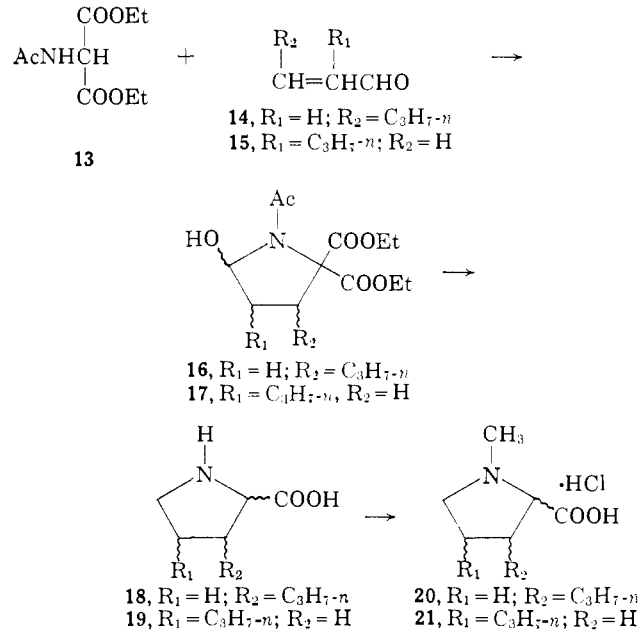
The amino acid fragment obtained from the hydrazinolysis of lincomycin was frequently partially or completely epimerized at the α-carbon atom. After conversion to the amides, the D and L isomers, **10** and **12**, were separated by chromatography.



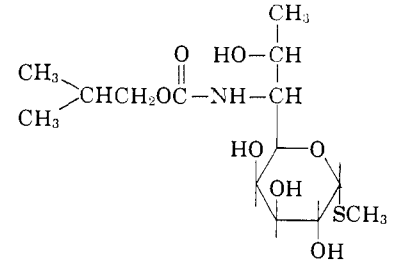
The nmr curves of diastereoisomers **10** and **11** (epimeric at the 4 position) differed from each other as did the curves of **10** and **12**. However, the curves of the mirror images **11** and **12** were identical as were those for **10** prepared from either synthetic amino acids **9** or natural amino acid **3**. Since the amides **10** prepared from either epimeric acids obtained by hydrazinolysis of lincomycin or from synthesis were identical, and since the synthetic amide was prepared from 4-keto-L-proline, we concluded that the *n*-propyl group was at position 4 and that the amino acid was in the L configuration. The isomers **11** and **12** which possessed identical nmr spectra, but optical rotations of opposite sign, were formulated as optical isomers though the absolute configuration of the propyl group was not as yet established. The absolute configuration of the *n*-propyl group was determined by degradation as will be described later.

Concurrently with the synthesis of 1-methyl-4-*n*-propyl-L-proline from 1-carbobenzoxy-4-keto-L-proline, total syntheses of this amino acid and 1-methyl 3-*n*-propylproline were investigated. The method of synthesis was similar to that published for racemic 4-methylproline.[6] Acetamidomalonic ester (**13**) was condensed with the appropriate aldehyde **14** or **15** to yield the cyclic intermediate **16** or **17**. This compound was not obtained pure, but was reduced and hydrolyzed to **18** or **19**. Reductive methylation of **18** and **19** gave racemic **20** and **21**. In neither case was racemic 3-*n*-propyl or 4-*n*-propyl-1-methylproline resolved though thin layer chromatography of the amide derivatives indicated that the *cis* and *trans dl* pairs could be separated by chromatography. The 4-substituted amides prepared from **21** showed two spots on thin layer chromatography which moved with the racemic acids obtained from lincomycin (**10** and **12**) and which separated from the 3-substituted amides prepared from **20**. This constituted additional evidence for 4 substitution in the hygric acid moiety of lincomycin.

The racemic acid **21** was coupled with the amine **2** using the mixed carbonic anhydride method. The crude product showed 12% of the activity of lincomycin when assayed microbiologically or by thin layer chromatography. No attempt was made to purify this material.

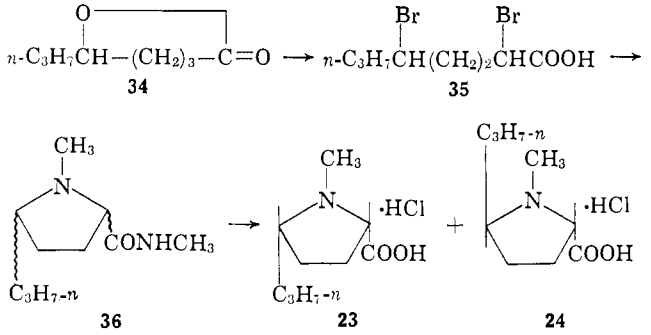


Under similar conditions racemic 1-methyl-3-*n*-propylproline (**20**) failed to condense with **2**, only the carbamate **22** being isolated from the reaction mixture.



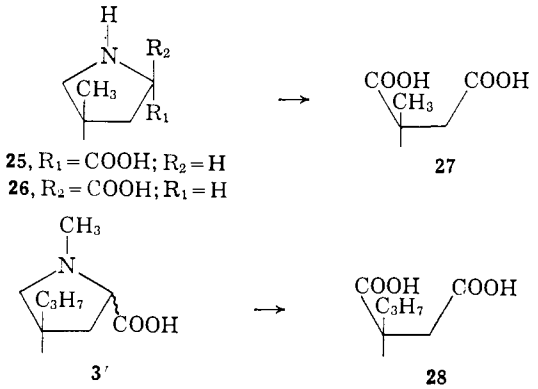
(6) J. S. Dalby, G. W. Kenner, and R. C. Sheppard, *J. Chem. Soc.*, 4387 (1962).

Racemic 1-methyl-5-*n*-propylproline was synthesized as outlined and separated into the racemic pairs **23** and **24**.

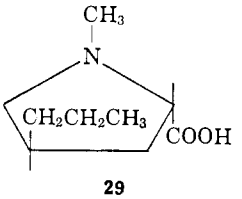


The nmr spectra of **23** and **24** differed sufficiently from that of the natural amino acid so as to exclude the 1-methyl-5-*n*-propylprolines (**23** and **24**) as lincomycin degradation products.

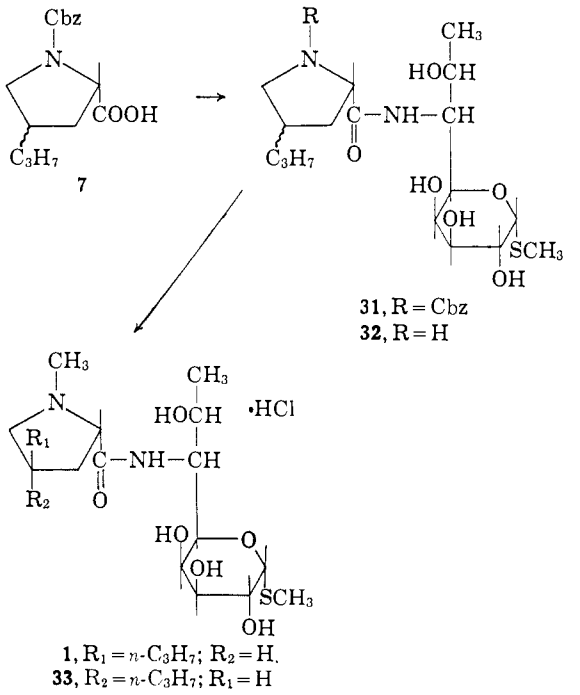
The stereochemistry at C-4 in 1-methyl-4-*n*-propylproline was determined by the use of a degradative method introduced by Neuberger in his classical work on the stereochemistry of hydroxyproline.[7] This method was recently employed in the determination of the absolute configuration of the 4-methyl group in 4-mentylproline (**25** and **26**).[8] In this case the oxidation of the diastereoisomers **25** and **26** gave D-(+)-methylsuccinic acid (**27**). Exhaustive oxidation of the racemized amino acid fragment (**3′**) from lincomycin afforded D-(+)-*n*-propylsuccinic acid (**28**).[9]



Since the stereorelationship between D-(+)-methyl and D-(+)-*n*-propylsuccinic acid is known,[10] we concluded that the propyl group of **3** is oriented similarly to the methyl group of **25** and **26**. That is, it is *trans* to the L-carboxyl. Therefore, the amino acid fragment from lincomycin is *trans*-1-methyl-4-*n*-propyl-L-proline (**29**).



Two partial syntheses of lincomycin (**1**) from 4-hydroxy-L-proline were achieved. In the first the *cis–trans* mixture of 1-methyl-4-*n*-propyl-L-proline (**9**) was coupled with methyl thiolincosaminide (**2**) to give a mixture of lincomycin (**1**) and its *cis* isomer **33**. In the first step of the second method, 1-carbobenzoxy-4-propylidene-L-proline (**6**) was hydrogenated to give **7**, a *cis* and *trans* mixture. The hydrogenation of the exocyclic double bond in 1-carbobenzoxy-4-propylidene-L-proline (**6**) over platinum oxide or platinum or palladium on the usual supports led to mixtures containing only a small percentage of the desired *trans* isomer. The palladium catalysts invariably also caused hydrogenolysis of the carbobenzoxy group. The high ratio of the *cis* isomer indicated that the hydrogen atoms were approaching the molecule from the side opposite the carboxyl group. Therefore, if the amino acid could be attracted to the catalyst by its carboxyl group, the hydrogen atoms should then approach from the carboxyl side forming a greater proportion of *trans* isomer. Such a situation may exist if the catalyst were deposited on a basic ion-exchange resin. Therefore, the use of platinum on an ion-exchange resin[11] was investigated and the hydrogenation product found to contain considerably more of the desired *trans* isomer of **7** (25–35%) in which the carbobenzoxy group remained intact.



The blocked amino acid **7** was coupled with methyl thiolincosaminide (**2**) in high yield, forming **31**. The blocking group was removed by hydrogenolysis and the resulting product **32** reductively methylated to yield a mixture of lincomycin (**1**) and its *cis* isomer **33**. From this mixture pure lincomycin (**1**) was isolated by chromatography. This material was identical in both its physical and microbiological properties with the natural antibiotic. The *cis* isomer **33** was also isolated as a pure compound. It possessed about one-half the antibacterial activity of lincomycin. This semisynthetic

(7) A. Neuberger, *J. Chem. Soc.*, 429 (1945).
(8) J. S. Dalby, G. W. Kenner, and R. C. Sheppard, *ibid.*, 4387 (1962).
(9) P. W. Clutterbuck, H. Raistrick, and F. Reuter, *Biochem. J.*, **31**, 987 (1937).
(10) S. Abrahamsson, S. Ställberg-Stenhagen, and E. Stenhagen, "Progress in the Chemistry of Fats and Other Lipids," Vol. VII, Part 1, R. T. Holman, Ed., Pergamon Press, Oxford, 1963, p 24.
(11) F. J. McQuillin, W. O. Ord, and P. L. Simpson, *J. Chem. Soc.*, 5996 (1963).

preparation of lincomycin confirmed the structure of the amino acid portion of lincomycin.

The partial synthesis of lincomycin from 4-hydroxy-L-proline has been adapted to the preparation of lincomycin analogs having variations in both the amino acid and sugar moieties.[12]

Experimental Section[13]

1-Carbobenzoxy-4-propylidene-L-proline Dicyclohexylamine Salt (6). A sodium hydride suspension (3.8 g) was warmed with 75 ml of dimethyl sulfoxide at 70–75° until reaction was completed. After cooling to 20°, 30.8 g of propyltriphenylphosphonium bromide was added. The resulting red solution was stirred for 30 min to ensure complete reaction. A solution of 5.2 g of **4**[14] in 15 ml of dimethyl sulfoxide was added over a period of 15 min. The resulting mixture was stirred for 20 min at 26° and then at 70° for 4 hr. The cooled reaction mixture was treated with 100 ml of 5% potassium bicarbonate and 100 ml of water and filtered. The filtrate was washed twice with 150-ml portions of ether and the ether discarded after back-extracting with bicarbonate solution. The aqueous solution was diluted with 200 ml of water, acidified, and extracted with three 200-ml portions of ether. The combined ether solution was washed with three 50-ml portions of saturated sodium bisulfite solution, then with water, and dried. The residue, 5.7 g, obtained after evaporation of the solvent, was dissolved in 18 ml of acetonitrile and treated with 2.8 ml of dicyclohexylamine. The crystalline salt, 5.2 g (55% yield), melted at 154–157°. The analytical sample prepared by three recrystallizations from acetonitrile melted at 164–166° and showed $[\alpha]D$ −8° (chloroform).

Anal. Calcd for $C_{25}H_{42}N_2O_4$: C, 71.45; H, 9.00; N, 5.95. Found: C, 71.77; H, 9.39; N, 5.91.

***trans*- and *cis*-4-*n*-Propyl-L-proline (8).** Ten grams of **6** was converted to the free base as described below in the preparation of **30**.

This oil was dissolved in 250 ml of methanol and shaken under hydrogen over 2 g of platinum on Dowex[11] for 4 hr. Palladium on carbon (2 g, 10%) was added and shaking continued for 2 hr. The catalyst was removed by filtration and the solvent distilled under reduced pressure. The residue was shaken with ether and 2.77 g (82.9%) of crystalline **8**, mp 220–223° dec, deposited. Three recrystallizations from methanol–acetone afforded the analytical sample, mp 232–234°, $[\alpha]D$ −63° (water).

Anal. Calcd for $C_8H_{15}NO_2$: C, 61.12; H, 9.26; N, 8.91. Found: C, 61.06; H, 9.46; N, 8.94.

***trans*- and *cis*-1-Methyl-4-*n*-propyl-L-proline Hydrochloride (9).** Formalin (4 ml) was added to 3.4 g of crude **8** from the previous step dissolved in 200 ml of methanol. The mixture was shaken under hydrogen for 2.5 hr at 45 psi pressure. The catalyst was removed by filtration and the solvent distilled. The residue was crystallized from methanol–ether–hydrogen chloride to afford 2.82 g of **9**, mp 200–202°. After several recrystallizations from methanol–ether the hydrochloride melted at 201–206° and gave $[\alpha]$ −60° (water).

Anal. Calcd for $C_9H_{18}NO_2Cl$: C, 52.04; H, 8.73; N, 6.75. Found: C, 51.72; H, 8.96; N, 6.44.

***cis*-Lincomycin Hydrochloride (33).** A mixture of 2.47 g of **9** and 7.6 ml of *n*-tributylamine in 80 cc of distilled acetonitrile was stirred until all of the solid had dissolved. The solution was cooled in an ice bath and 1.64 g of isobutyl chloroformate slowly added. A solution of 3.0 g of methyl thiolincosaminide in 40 ml of water was added. The reaction mixture was stirred for 1 hr in the ice bath and 3 hr at 26°. The acetonitrile was distilled under vacuum. The residue was diluted with 20 ml of water and extracted twice with ether. The aqueous solution was lyophilized. The residue was triturated several times with chloroform and this solution chromatographed over Florisil,[15] using an elution system of Skellysolve B[16]–ethyl acetate with increasing amounts of methanol. The fractions which showed material in the lincomycin area by tlc were combined. This oil (0.86 g) was dissolved in dilute hydrochloric acid and the crude hydrochlorides **33** and **1** precipitated with acetone. The yield was 480 mg (9.04%). Tlc (ethyl acetate–acetone–water, 8:5:1) showed a major spot moving slightly slower than lincomycin and also a weak lincomycin spot. Bioassay indicated 37% lincomycin. Bioautography showed activity in the lincomycin area.

Several recrystallizations of the crude hydrochloride afforded an analytical sample, mp 147–150°, of *cis*-lincomycin hydrochloride (**33**) containing only a trace of lincomycin.

Anal. Calcd for $C_{18}H_{35}ClN_2O_6S$: C, 48.80; H, 7.96; N, 6.32. Found (corrected for 9.47% H_2O): C, 49.15; H, 7.80; N, 6.39.

***cis*-1-Methyl-4-*n*-propyl-L-proline Amide (11) and *trans*-1-Methyl-4-*n*-propyl-L-proline Amide (10).** A mixture of 3.09 g of **9**, 9.5 ml of tri-*n*-butylamine, 100 ml of acetonitrile, and 40 ml of acetone was stirred until solution was complete. To this solution, cooled to 10°, there was added 2.05 ml of isobutyl chloroformate. The reaction mixture was stirred for 30 min in an ice bath after which time 15 ml of ammonium hydroxide was added. Stirring was continued for 2 hr. The solvent was distilled under vacuum. The residue was acidified and extracted with ether. After neutralizing the aqueous solution, it was extracted with methylene chloride. Chromatography of this product over silica gel[15] using 80% aqueous acetone as the eluent gave two fractions, one showing an enrichment of **10**, the other almost pure **11**. The **10** component-rich fraction (359 mg) was rechromatographed to yield a small fraction of fairly pure **10**, the other fractions being mixtures of **10** and **11**. The latter fractions were again rechromatographed and the resulting fractions recombined on the basis of tlc. The fractions containing the purest **10** were combined and recrystallized from Skellysolve B to give 10 mg of **10**, estimated to be about 85% pure on the basis of tlc. This material showed $[\alpha]D$ −93° (water). Its nmr was almost identical with **10** isolated from natural 4-propylhygric acid.

In another experiment the original crystalline product was recrystallized for analysis from ethyl acetate–Skellysolve B. Tlc showed it to be almost pure **11**, mp 113.5–115.5°, $[\alpha]D$ −104° (water).

Anal. Calcd for $C_9H_{17}O_2N$: C, 63.49; H, 10.66; N, 16.46. Found: C, 63.41; H, 10.76; N, 16.28.

***trans*-1-Methyl-4-*n*-propyl-L-proline Amide (10) and *cis*-1-Methyl-4-*n*-propyl-D-proline Amide (12) from Hydrazinolysis Cleavage Product.** Partially racemic 1-methyl-4-*n*-propylproline was converted to the mixture of isomeric amides in the same manner as described for the preparation of racemic 1-methyl-3-*n*-propylproline amide. Recrystallization of the crude product from Skellysolve B gave a 65% yield of material, mp 86–86.5°, $[\alpha]D$ +10° (water).

Anal. Calcd for $C_9H_{18}NO_2$: C, 63.49; H, 10.66; N, 16.46. Found: C, 63.35; H, 10.92; N, 16.28.

A thin layer chromatograph of the above material on silica gel, using an acetone–water (8:2) irrigating system, disclosed the presence of two compounds of very similar mobility. Separation was effected *via* chromatography over silica gel using acetone–water (8:2) for elution. The faster moving component was identified as the L-*trans* amide and melted at 117–118° after recrystallization from Skellysolve B; $[\alpha]D$ −93° (water).

Anal. Calcd for $C_9H_{19}NO_2$: C, 63.49; H, 10.66; N, 16.46. Found: C, 63.57; H, 10.73; N, 16.71.

The more polar component, the D-*cis* amide, $[\alpha]D$ +103° (water), melted at 114–115° after recrystallization from Skellysolve B.

1-Acetoxy-2,2-carbethoxy-4-propyl-5-hydroxypyrrolidine (17). A mixture of 5.6 g of 2-propylacrolein (**15**),[18] 10 g of diethyl acetamidomalonate, and 0.25 ml of 26% sodium methoxide–methanol solution in 150 ml of benzene was stirred for 3 hr at ambient temperature. The solution was neutralized with a few drops of acetic acid, clarified by filtration, and evaporated. This material did not crystallize and was used in the following step.

Racemic 4-*n*-Propylproline Hydrochloride (19). The pyrrolidine **17** from above was suspended in 127 ml of water and 127 ml of concentrated hydrochloric acid, and 22.8 g of granulated tin added.

(12) For a preliminary report, see B. J. Magerlein, Abstracts of Papers, 5th Interscience Conference on Antimicrobial Agents and Chemotherapy, and 11th International Congress of Chemotherapy, Washington, D. C., Oct 17–21, 1965, p 17.

(13) Melting points were taken in Pyrex capillaries and are corrected. Infrared spectra were recorded on a Perkin-Elmer Model 21 spectrophotometer equipped with sodium chloride optics. Nuclear magnetic resonance spectra were run on a Varian, high-resolution, 60-Mc instrument; measurements are expressed in parts per million downfield from tetramethylsilane used as an internal standard.

(14) A. A. Patchett and B. Witkop, *J. Am. Chem. Soc.*, 79, 185 (1957).

(15) Synthetic magnesia–silica gel manufactured by the Floridin Co., Pittsburgh, Pa.

(16) A saturated hydrocarbon fraction, bp 60–71°, available from Skelly Oil Co., Kansas City, Mo.

(17) Silica gel 0.05–0.20 mm for chromatography, E. Merck A. G. Distributors, Brinkmann Instruments, Inc., Westbury, N. Y.

(18) M. B. Green and W. J. Hickinbottom, *J. Chem. Soc.*, 3262 (1957).

After refluxing for 1 hr, the metal was removed by filtration and the solvent evaporated under vacuum. The solution was saturated with hydrogen sulfide, and the precipitated solids were separated. The filtrate was treated repeatedly in this manner. The reaction mixture was lyophilized. The residue was dissolved in a small amount of water, extracted with ether, and again lyophilized. It was then dried at 55° under vacuum. The residue, 14.7 g, was crystallized from acetonitrile affording 1.62 g of **19**, mp 162–165°.

Several recrystallizations from methanol–ether of material prepared in a similar manner afforded an analytical sample, mp 165–168°.

Anal. Calcd for $C_8H_{15}NO_2 \cdot HCl$: C, 49.61; H, 8.33; N, 7.23. Found: C, 49.24; H, 8.13; N, 7.53.

Racemic 1-Methyl-4-propylproline Hydrochloride (21). In the manner described for the preparation of **20**, 1.1 g of **19** was methylated to obtain 620 mg of **21**, mp 164–166°. Recrystallization from methanol–ether gave an analytical sample, mp 165–167°.

Anal. Calcd for $C_9H_{17}NO_2 \cdot HCl$: C, 52.04; H, 8.73; N, 6.75. Found: C, 51.74; H, 8.48; N, 7.13.

Racemic 1-Methyl-4-*n*-propylprolylamide. In the manner described for the preparation of **10** and **12**, 1.0 g of **21** was converted to the racemic amides, mp 86–89°, in 90% yield. An analytical sample prepared from Skellysolve B melted at 84–85°.

Anal. Calcd for $C_9H_{19}NO_2$: C, 63.49; H, 10.66; N, 16.46. Found: C, 63.53; H, 10.64; N, 16.48.

Racemic Lincomycin. In the manner described for the preparation of **33**, racemic **21** was condensed with methyl thiolincosaminide (**2**) to give a crude product which possessed 11% of the antibacterial activity of lincomycin and showed about the same amount of lincomycin by thin layer chromatography.

1-Acetoxy-2,2-carbethoxy-3-propyl-5-hydroxypyrrolidine (16). In the manner described above, 2-hexenal[19] (**14**) and diethyl acetamidomalonate were condensed to afford **16**, mp 101–102°, in a 62% yield after recrystallization from acetone–ether.

Anal. Calcd for $C_{15}H_{25}NO_6$: C, 57.13; H, 7.99; N, 4.44. Found: C, 57.07; H, 8.02; N, 4.72.

Racemic 3-*n*-Propylproline Hydrochloride (18·HCl). Acidic hydrolysis of **16**, as described in the preparation of **17**, afforded a 26% yield of **18**, mp 165–168°, after recrystallization from ethanol–ether.

Anal. Calcd for $C_8H_{16}ClNO_2$: C, 49.61; H, 8.33; N, 7.23; Cl, 18.31. Found: C, 49.84; H, 8.30; N, 7.49; Cl, 18.09.

Racemic 3-*n*-Propylproline (18). A mixture of 880 mg of 3-*n*-propylproline hydrochloride (**18**·HCl), 2.0 g of silver carbonate, and 10 ml of water was stirred at 25° for 0.5 hr and then warmed on a steam bath for 30 min and filtered. The filtrate was evaporated under vacuum and the residue recrystallized from ethanol–ethyl acetate to a melting point of 229–230°. The yield of once recrystallized material was 600 mg (85% yield).

Anal. Calcd for $C_8H_{15}NO_2$: C, 61.12; H, 9.62; N, 8.91. Found: C, 61.45; H, 9.60; N, 8.95.

Racemic 1-Methyl-3-*n*-propylproline (20). A mixture of 14 g of 3-*n*-propylproline (**18**), 11.8 ml of formaldehyde, 200 ml of methanol, and 2.0 g of 10% Pd–C catalyst was hydrogenated on a Parr hydrogenator at 35 psi hydrogen pressure. A yield of 11.6 g (76%) of product was obtained, melting at 202–203°, after recrystallization from ethanol–ethyl acetate.

Anal. Calcd for $C_9H_{17}NO_2$: C, 63.12; H, 10.01; N, 8.18. C, 62.65; H, 10.02; N, 8.42.

Racemic 1-Methyl-3-*n*-propylprolylamide. To a stirred mixture of 1.03 g of racemic 1-methyl-3-*n*-propylproline (**20**), 1.68 ml of triethylamine, and 69 ml of dry acetonitrile at 0° was added 0.58 ml of ethyl chloroformate. The reaction mixture was maintained at 0–10° for 0.5 hr and 6 ml of concentrated NH_4OH then added. After standing 18 hr at 25°, the reaction mixture was evaporated to dryness under vacuum and the residue dissolved in 50 ml of water, acidified with HCl, and extracted with four 50-ml portions of methylene chloride. The methylene chloride extracts were discarded and the aqueous phase was neutralized and extracted with three 50-ml portions of methylene chloride. The methylene chloride phases were combined and evaporated and the residue was recrystallized from Skellysolve B to a melting point of 99–100°. A 60% yield of product was obtained.

Anal. Calcd for $C_9H_{19}N_2O$: C, 63.49; H, 10.66; N, 16.46. Found: C, 63.60; H, 10.85; N, 16.22.

This material showed only one spot when chromatographed on silica gel using a methyl ethyl ketone–acetone–water system (75:25:10).

Attempted Coupling of Racemic 1-Methyl-3-*n*-propylproline (20) with Methyl Thiolincosaminide. To a mixture of 60 ml of distilled acetonitrile, 1.68 ml of triethylamine, and 1.03 g of amino acid **20** cooled at 0° was added 0.87 ml of isobutyl chloroformate. After stirring at 0° for 0.5 hr, 1.52 g of methyl thiolincosaminide dissolved in 20 ml of water was added. Stirring was continued for a period of 60 hr at 25°. The reaction mixture was evaporated to dryness and the residue chromatographed over silica gel, eluting with acetone. The fast-moving material was isolated (140 mg) and recrystallized from acetone–Skellysolve B to a melting point of 186–187°. The infrared and nmr data support the carbamate structure **22**.

Anal. Calcd for $C_{14}H_{27}NO_7S$: C, 47.57; H, 7.70; N, 3.96; S, 9.07. Found: C, 47.73; H, 7.84; N, 4.03; S, 9.25.

Ethyl 2,5-Dibromooctanoate (35). At 0° 13 g of 5-caprylolactone (**34**) was added to 10.5 ml of phosphorus tribromide followed by 7.1 ml of bromine. After stirring at ambient temperature for 18 hr, 1 ml of phosphorus tribromide and 6 ml of bromine were added. The reaction mixture was heated at reflux for 24 hr. To the cooled solution was added 30 ml of absolute ethanol and the mixture heated at reflux for 1.5 hr. The solvents were removed under vacuum and the residue was dissolved in benzene. This solution was washed free of bromide ion, dried, and concentrated. The yield of **35**, bp 125–135° (0.8 mm), was 15–20 g (43–57% yield).

Anal. Calcd for $C_{10}H_{18}Br_2O_2$: C, 36.38; H, 5.49; Br, 48.43; O, 9.70. Found: C, 35.43; H, 5.37; Br, 48.75; O, 9.81.

Racemic 1-Methyl-5-*n*-propylproline N-Methylamides (36). A 14.85-g quantity of ethyl 2,5-dibromooctanoate (**35**) was condensed with 17.4 g of methylamine in 85 ml of methanol by heating at 140° for 4 hr in an autoclave. The reaction mixture was filtered and evaporated *in vacuo* to dryness. The residue was dissolved in chloroform and filtered to remove methylamine hydrobromide. The filtrate was evaporated to dryness, and the residue was distributed countercurrently for 200 transfers using the solvent system ethyl acetate–ethanol–cyclohexane–water (1:1:1:1). Two main peaks were noted on analysis by determination of solids. The first, in tubes 80–107, was pooled and evaporated to dryness. The partially crystalline residue was recrystallized from Skellysolve B to give 1.8 g of product.

Anal. Calcd for $C_{10}H_{20}N_2O$: C, 65.18; H, 10.94; N, 15.21. Found: C, 65.86; H, 11.27; N, 14.54.

The second peak in tubes 118–150 was pooled and evaporated to dryness. The residue crystallized in the refrigerator, but melted at room temperature. No suitable solvent for recrystallization was found, so this material was used directly in the next step.

***cis*- and *trans*-1-Methyl-5-*n*-propylproline Hydrochloride (23 and 24).** A 250-mg sample of the crystalline methylamide of 1-methyl-*n*-propylproline (**36**) was heated at reflux with 10 ml of 6 *N* HCl for 4 hr. The solution was evaporated to dryness *in vacuo*. The residue was dissolved in water and Dowex 2 (OH^-) was added to about pH 10. The resin was collected and eluted with dilute hydrochloric acid. The eluate was evaporated to dryness, and the residue was dissolved in a small amount of anhydrous ethanol. Ether was added until the solution was cloudy, and the mixture was cooled. A small amount of yellow oil separated. The supernatant was decanted and diluted with several volumes of ether. Crystals formed slowly on cooling. From two such runs, 143 mg of crystalline material was obtained. Recrystallization from acetonitrile–ether gave 95 mg of hydrochloride.

Anal. Calcd for $C_9H_{18}ClNO_2$: C, 52.04; H, 8.73; N, 6.74; O, 15.41; Cl, 17.07. Found: C, 51.12; H, 8.45; N, 7.03; O, 14.85; Cl, 16.55.

About 800 mg of the lower melting isomer was hydrolyzed by heating under reflux with 40 ml of 6 *N* HCl for 4 hr. The product was isolated as described above to give 107 mg of recrystallized amino acid hydrochloride.

Anal. Found: C, 51.82; H, 9.33; N, 7.08; O, 18.5; Cl, 16.90.

D-(+)-*n*-Propylsuccinic Acid (27) from Oxidation of Partially Racemic 1-Methyl-4-*n*-propylproline. A solution of 7.44 g of partially racemized 1-methyl-4-*n*-propylproline hydrochloride in 100 ml of water containing 36 ml of 1 *N* sodium hydroxide was added slowly with vigorous stirring to 28 g of potassium permanganate in 550 ml of water. The temperature of the mixture was maintained at 25 ± 2° by occasional cooling. When most of the acid was added the permanganate color was no longer present. Therefore, 2 g of the additional permanganate was added. The reaction mixture was filtered after the addition of filter aid. The purple color of the filtrate was discharged with excess sodium bisulfite and

(19) R. I. Hoaglin and D. H. Hirsh, *J. Am. Chem. Soc.*, 71, 3468 (1949).

the solution refiltered. The clear filtrate was acidified and lyophilized. The residue from lyophilization was shaken with absolute alcohol–ether (3:1) and filtered. The filtrate was evaporated to yield 4.4 g of oil. This oil was chromatographed over 125 g of silica gel using a mixture of solvents for elution made up of benzene–methanol–acetic acid (10:2:1). The fractions thus obtained were checked by thin layer chromatography (same system) using *dl*-propylsuccinic acid[20] as the control. Those fractions showing only material moving with the control were combined (1.83 g) and crystallized from benzene–Skellysolve B several times. The crystalline product, 280 mg, melted at 101.5–103.5° and rotated at +23° (water).

Anal. Calcd for $C_7H_{12}O_4$: C, 52.49; H, 7.55. Found: C, 52.48; H, 7.67.

***cis*- and *trans*-1-Carbobenzoxy-4-*n*-propyl-L-proline (30).** The amine salt (**6**, 10 g) was shaken with ether and 2% potassium hydroxide. The aqueous layer was separated and acidified. Extraction with methylene chloride led to the isolation of 6 g of oily acid. A mixture of 2 g of this oil and 800 mg of platinum on Dowex-1 catalyst[10] in 50 ml of methanol was shaken under 40 psi hydrogen pressure for 17 hr. The catalyst was removed by filtration and the solvent distilled *in vacuo*, leaving a residue of 2 g of oily **30**. Thin layer chromatography, using a methanol–5% ammonium hydroxide system and permanganate–periodate indicator spray, indicated that the double bond was hydrogenated. Ninhydrin gave a negative test. This product resisted crystallization and was used without purification in the next step.

***cis*- and *trans*-Methyl N-(1′-Carbobenzoxy-4′-*n*-propyl-L-prolyl)-thiolincosaminide (31).** To a solution of 7.5 g of **30** and 4.25 ml of triethylamine in 700 ml of distilled acetonitrile cooled to 0° there was added 4.16 ml of isobutyl chloroformate in 5 ml of acetonitrile. The mixture was stirred at 0° (±5°) for 15 min. A solution of 7 g of methyl thiolincosaminide (**2**) in 100 ml of water was added rapidly. The resulting solution was stirred at 0° for 1 hr, the cooling bath removed, and stirring continued for another hour. The acetonitrile was removed by distillation under vacuum leaving a partially crystalline residue. The mixture was cooled to 10° and filtered. After drying at 55° under vacuum the crystalline product weighed 10.5 g and melted at 191–194°. *In vitro* assay *vs. S. lutea* showed <1% the antibacterial activity of lincomycin. This material was recrystallized twice from ethyl acetate containing a few drops of water to afford the analytical sample, mp 197–203°, $[\alpha]D$ +111° (MeOH).

Anal. Calcd for $C_{23}H_{38}N_2O_6S$: C, 57.01; H, 7.27; N, 5.32; S, 6.09. Found: C, 56.93; H, 7.45; N, 5.35; S, 6.03.

***cis*- and *trans*-Methyl N-(4-Propyl-L-prolyl)thiolincosaminide Hydrochloride (32·HCl).** A solution of 8.95 g of **31** in 200 ml of methanol was shaken over 2 g of 10% Pd–C under 40 psi hydrogen pressure for 6 hr. The catalyst was removed by filtration and the solution concentrated under vacuum. The residue was dissolved in 75 ml of 0.5 *N* hydrochloric acid and 250 ml of water with warming to about 35°. Dilution with 1500 ml of acetone precipitated **32**·HCl which was collected by filtration. The crystals, dried at 55° under vacuum, weighed 6.0 g (82.3% yield) and melted at 202–205°. The rotation was +151° (H_2O).

Anal. Calcd for $C_{17}H_{32}N_2O_6S \cdot HCl$: C, 47.57; H, 7.75; N, 6.57; S, 7.47. Found (corrected for 3.31% water): C, 47.67; H, 7.72; N, 6.53; S, 7.14.

In one experiment the free base crystallized from acetone. It melted at 178–180°.

Anal. Calcd for $C_{17}H_{32}N_2O_6S$: C, 52.02; H, 8.22; N, 7.14. Found: C, 51.97; H, 8.01; N, 7.00.

Lincomycin Hydrochloride (1) and *cis*-Lincomycin Hydrochloride (33). Two grams of **32**·HCl, 2.4 ml of formalin, and 800 mg of 10% palladium on carbon in 200 ml of methanol was shaken under 40 psi hydrogen pressure for 5 hr. The catalyst was removed by filtration. Triethylamine (1 ml) was added and the solution evaporated. The residue was chromatographed twice over silica gel using for elution a solvent mixture of ethyl acetate–acetone–water (8:5:1). The various fractions were monitored by thin layer chromatography on silica gel using the same solvent system and fractions showing only **1** and **33** combined. The crude **1** (free base) fraction weighed 320 mg. It was dissolved in dilute hydrochloric acid and **1**·HCl precipitated by the addition of acetone. The yield of **1**·HCl was 290 mg. After two recrystallizations from the same solvent it melted at 155–157° dec. This material had identical spectra with lincomycin hydrochloride by infrared and nmr. It possessed full antibacterial activity when compared with lincomycin hydrochloride.

The slower moving fraction, 570 mg, from above was similarly converted to its hydrochloride. After recrystallization from acetone–water it melted at 138–145° dec and was identical in the infrared and nmr with *cis*-lincomycin hydrochloride (**33**) prepared as described above.

(20) P. A. S. Smith and J. P. Horwitz, *J. Am. Chem. Soc.*, 71, 3418 (1949).

The Morphine–Thebaine Group of Alkaloids. IX.[1] The Reaction of Thebaine with Magnesium Iodide

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Abstract: The reaction of thebaine with anhydrous magnesium iodide has been shown to give the iminium salt VIII, the structure of which has been deduced from its oxidation to 4-methoxyphthalic acid, its hydrolysis and cyclodehydration to thebenine (II), and its reduction to neodihydrothebaine (VII, R = H). The structure of neodihydrothebaine has been confirmed by degradation and spectral studies. Hydrolysis and recyclization of the iminium salt VIII has been found to give an enamine, kryptothebaine, which is clearly not the enamine I and is assigned structure IV.

The reaction of thebaine (III) with anhydrous magnesium iodide in ether and benzene has previously been shown[2] to give a product containing magnesium and iodine, to which the iminium salt structure VIII was assigned on the basis of its conversion into phenyldihydrothebaine (VII, R = Ph) on treatment with phenylmagnesium bromide. This assignment of structure is now supported by the reduction of the salt to neodihydrothebaine (VII, R = H), by its oxidation to 4-

(1) Part VII: K. W. Bentley, J. C. Ball, and J. P. Ringe, *J. Chem. Soc.*, 1963 (1956); the paper by K. W. Bentley and S. F. Dyke, *ibid.*, 2574 (1959), is now regarded as part VIII of this series.

(2) K. W. Bentley and R. Robinson, *ibid.*, 947 (1952).