Product Analysis for Acetolysis of 13b. The solvolysis of 660 mg of **13b** under the conditions described above was stopped after 8 half-lives. The solvolysis mixture was cooled and poured into a mixture of ice and saturated sodium carbonate solution. The resultant acetates were extracted with ether and dried over anhydrous magnesium sulfate. The drying agent was removed by filtration, and the filtrate was concentrated under reduced pressure. The ethereal concentrate was added to a stirred slurry of lithium aluminum hydride (0.75 g) in dry ether (50 ml). After stirring for 1 hr, the reaction mixture was hydrolyzed by dropwise addition of water. Removal of the inorganic salts by filtration followed by evaporation of the solvent under reduced pressure and distillation of the residue at 12 mm with a pot temperature of 90–115° gave 152 mg (54%) of a mixture of **12a** and **13a**. Vapor phase chromatography on a column of 10% Carbowax 20M on Firebrick at 160° indicated the presence of three components. The relative amounts of these components were 93% of **12a** (retention time 8.8 min) and 7% of **13a** (retention time 7.5 min). An infrared spectrum of this mixture was identical with a spectrum of the mixed authentic alcohols, **12a** and **13a**; the third component (retention time 8.3 min) was estimated to comprise not more than 5–10% of the mixture.

Product Analysis for Acetolysis of 12b. The solvolysis of 660 mg of **12b** was stopped after 8 half-lives. Work-up and isolation of the solvolysis products was the same as that described above for the solvolysis of **13b**. Vapor phase chromatography of the product mixture on a column of 10% Carbowax 20M at 160° revealed that the relative composition was 94% of **13a**, and 6% of **12a**. An infrared spectrum of this mixture was identical with a spectrum of this composition of authentic **12a** and **13a**. A minor component was not identified.

Product Analysis for Acetolysis of 9b. The solvolysis of 660 mg of **9b** was allowed to proceed for 8 half-lives. Work-up and isolation of the solvolysis products was the same as that described above for the solvolysis of **13b**. Vapor phase chromatographic analysis of the product mixture on a column of 20% Carbowax 20M at 185° indicated the presence of 9% of **9a** (retention time 7.0 min) and 91% of **8a** (retention time 10.3 min). An infrared spectrum of this mixture was identical with a spectrum of the mixed authentic alcohols, **8a** and **9a**.

Product Analysis for Acetolysis of 8b. The solvolysis of 660 mg of **8b** was allowed to proceed for 8 half-lives. Work-up and isolation of the solvolysis products was the same as that described above for the solvolysis of **13b**. Vapor phase chromatographic analysis of the product mixture on a column of 10% Carbowax 20M at 160° indicated the relative composition of the mixture to be 84% of **9a** (retention time 4.5 min) and 16% of **8a** (retention time 7.85 min). An infrared spectrum of this mixture was identical with a spectrum of the mixed authentic alcohols, **8a** and **9a**. A minor component with a 5.5-min retention time was not identified.

Structure and Synthesis of the Major Components in the Hairpencil Secretion of a Male Butterfly, *Lycorea ceres ceres* (Cramer)[1]

Jerrold Meinwald and Yvonne C. Meinwald

Contribution from the Department of Chemistry, Cornell University, Ithaca, New York. *Received December 16, 1965*

Abstract: Males of the tropical butterfly, *Lycorea ceres ceres* (Cramer), possess elaborate retractable organs, the hairpencils, which appear to play an important role in courtship. As a first step toward identifying a new pheromone from this source, hairpencils were removed from live males and extracted with organic solvents. The extract was found to consist of three major components, proved to be 2,3-dihydro-7-methyl-1H-pyrrolizin-1-one (**5**), cetyl acetate (**9**), and *cis*-vaccenyl acetate (**16**) on the basis of chemical and physical evidence. These structures were confirmed by independent syntheses.

In a stimulating study of the courtship behavior of the Queen butterfly [*Danaus gilippus berenice* (Cramer)], Brower, *et al.*,[2] call attention to many significant contributions to the development of biology which have resulted from investigating the sexual behavior of the Lepidoptera. One field that has received particular impetus in this way is that of chemical communication, an area of "molecular biology" which is, nevertheless, still in its infancy.[3] The present paper describes chemical studies which may ultimately prove relevant to this growing area of research.

It has long been known that male butterflies throughout the tropical subfamily Danainae generally possess a pair of elaborate organs, called hairpencils, which can be extruded from the end of the abdomen.[4] In the Queen butterfly, the male has been observed to brush the hairpencils across the anterior end of the female in flight. This treatment appears to induce the female to settle on available herbage. After continued "hairpencilling," copulation occurs. The hairpencils and their associated glandular cells therefore seem to play an important role in courtship, and are thought to be concerned with the production and/or dissemination of a "sexual scent."[2] The exact biological role of such scents, which remarkably enough are often agreeable to man, remains to be defined. It has been speculated that they may form part of "a chemical language that prevents interspecific hybridization in nature."[2] In spite of the obvious interest such a function would have, no study of the chemical nature of these hairpencil secretions has been reported. We now present the results of a chemical study of the hairpencil secretion obtained from the Trinidad butterfly *Lycorea ceres ceres* (Cramer),

(1) The partial support of this work by National Institutes of Health Research Grant AI-2908 and Training Grant 5TI-GM-A34-02 is acknowledged with pleasure.

(2) L. P. Brower, J. V. Z. Brower, and F. P. Cranston, *Zoologica*, **50**, 1 (1965).

(3) For an excellent recent review of this subject, see E. O. Wilson and W. H. Bossert, *Recent Progr. Hormone Res.*, **14**, 673 (1963).

(4) F. Müller speculated on the function of these organs as long ago as 1877 (see ref 2).

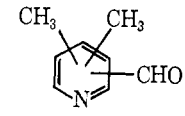
a member of the Lycoreini tribe of the Danainae subfamily.[5]

Isolation and Preliminary Characterization of Hairpencil Components. Several hundred male *Lycorea* were captured in Trinidad[6] and mailed alive in small envelopes to Ithaca, N. Y. The hairs were carefully removed from their extruded hairpencils and extracted directly with methylene chloride or carbon disulfide.[7] The resultant solution showed strong infrared absorption bands at 3.45, 3.52, 5.77, 5.92, and 8.10 μ. Upon glpc analysis using a 5% SE 30 column at 180°, the solution showed the presence of three major components with retention times of 1.7, 10.5, and 20 min. Chromatography on a silicic acid column of the secretion extracted from 100 males led to the isolation of about 28 mg of an *ester* fraction and about 10 mg of a *carbonyl* fraction. The ester fraction was characterized by a single carbonyl band in its infrared spectrum (CS_2) at 5.77 μ, along with other maxima at 3.42, 3.52, and 8.10 μ. Glpc analysis of this fraction showed the presence of the two longer retention time components, and these could be separated preparatively, using a 5% SE 30 column at 200°, to give 4.5 mg of ester I (5 min) and 8.5 mg of ester II (8.5 min).

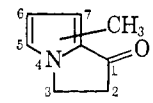
The carbonyl component was first obtained as a viscous oil which showed chiefly the short retention time peak upon gas chromatographic analysis. This material crystallized upon storage in the refrigerator and could be purified by vacuum sublimation until it showed mp 74–75°. It had characteristic infrared absorption maxima at 3.4, 5.95, and 6.45 μ; in the ultraviolet it showed λ_{max}^{EtOH} 288 mμ (log ϵ 4.22, based on a mass spectrally determined molecular weight[8]), indicative of an extended, conjugated system.

A more efficient preparative technique for separating these three components proved to be preferential vacuum sublimation of the crystalline carbonyl compound followed by glpc separation of the residual ester mixture. In this way, the tedious column chromatography could be avoided, and losses could be reduced.

Structure and Synthesis of the Carbonyl Component. The crystalline carbonyl compound, isolated as described above, appeared homogeneous on the basis of its gas chromatogram and its behavior on a silica gel tlc plate. It had a sweet odor, somewhat reminiscent of vanillin. The infrared and ultraviolet spectra were suggestive of a conjugated carbonyl group, and the formation of a reddish 2,4-dinitrophenylhydrazone supported this hypothesis. The mass spectrum[8] of this component had its base peak (also the parent peak) at *m/e* 135, suggesting a nitrogen-containing molecule, with 107 (M − 28) and 79 (M − 56) as prominent fragments. Assuming from the infrared evidence the presence of one oxygen atom, the molecular formula C_8H_9ON is indicated. From 107 down, the mass spectrum was rather similar to those of the ethylpyridines (C_7H_9).[9] Since M − 15 is a more important fragment in the ethylpyridines than in the compound under examination, a dimethylpyridine aldehyde structure (**1**) seemed possible.



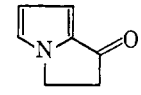
1



2

When a large enough sample (*ca.* 6 mg) of the nitrogenous carbonyl compound was obtained pure, a 100-Mc nmr spectrum was taken.[10] The gross features of this spectrum immediately ruled out all structures of the general type **1**, since there is no absorption in the aldehyde region and only *one* methyl group (singlet at τ 7.80) rather than two can be discerned. Aside from the three protons of methyl group, the pair of two-proton triplets ($J \cong 6.5$ cps) at τ 7.16 and 5.87 are indicative of the presence of a $-CH_2-CH_2-$ grouping in a relatively deshielded environment. Finally, a pair of poorly resolved one-proton doublets ($J \cong 2.5$ cps) at τ 3.91 and 3.31 are characteristic of two coupled aromatic or olefinic protons. In this way, all eight protons indicated by the mass spectral molecular weight are accounted for in three groups of nmr absorptions, each of which appear insulated from the other two.

Combining these structural features with those derived from the previously summarized physical data leads directly to a 2,3-dihydro-*x*-methyl-1H-pyrrolizin-1-one structure (**2**). Fortunately, the parent bicyclic heterocyclic ketone, 2,3-dihydro-1H-pyrrolizin-1-one (**3**), is a well-known compound, and a sample could be readily prepared.[11,12] The ultraviolet spectrum of **3** (λ_{max}^{EtOH} 288 mμ (log ϵ 4.30)) is in excellent agreement with that of the natural product. A small sample of **3** was prepared, and its nmr spectrum revealed the expected



3

pair of two-proton triplets ($J \cong 6.5$ cps) at τ 7.05 and 5.75, very close to those seen in the natural product. In its mass spectrum, **3** yielded important peaks at *m/e* 121, 93 (M − 28), and 63 (M − 56). On the basis of the bicyclic, heterocyclic structures, this fragmentation pattern is readily rationalized as involving loss of either CO or C_2H_4 (M − 28), and loss of both CO and C_2H_4 (M − 56). In summary, the close parallel between the properties of model substance **3** and those of the natural ketone lend very strong support to partial structure **2**.

A tentative decision among the three possible positions (C_5, C_6, or C_7) for the methyl group in **2** could be made on the basis of its nmr spectrum. Thus the spin–spin coupling constant for an α and β proton on a pyrrole nucleus (**4**) is reported[13] to be *ca.* 2.5 cps, while

(5) For a preliminary communication of part of these results, see J. Meinwald, Y. C. Meinwald, J. W. Wheeler, T. Eisner, and L. P. Brower, *Science*, **151**, 583 (1966).

(6) We are indebted to Dr. Jocelyn Crane, H. Croze, T. Pliske, and Dr. Lincoln Brower for their considerable effort in collecting these butterflies.

(7) These operations were performed by Dr. Thomas Eisner. Dead males were essentially useless, since there was no convenient way to remove the hairpencils, and extraction of the entire abdomens gave very complex mixtures. Preliminary work in characterizing this secretion was carried out by Dr. J. W. Wheeler.

(8) We wish to express our gratitude to Drs. A. F. Thomas and B. Willhalm for their mass spectral examination of all of the key compounds associated with this program.

(9) These spectra are reproduced in K. Biemann, "Mass Spectrometry," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, p 135.

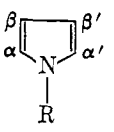
(10) We are grateful to Dr. Ross Pitcher of Varian Associates for taking this spectrum.

(11) G. R. Clemo and G. R. Ramage, *J. Chem. Soc.*, 53 (1931).

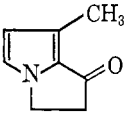
(12) A. D. Josey and E. L. Jenner, *J. Org. Chem.*, **27**, 2466 (1962). We are indebted to Dr. Josey for a sample of the semicarbazone of **3**.

(13) R. F. M. White in "Physical Methods in Heterocyclic Chemistry," Vol. 2, A. Katritzky, Ed., Academic Press Inc., New York, N. Y., 1963.

a β,β' coupling is *ca.* 3.5 cps and an α,β' coupling 1.5 cps. With an observed splitting of *ca.* 2.5 cps in the



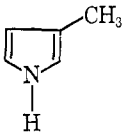
4



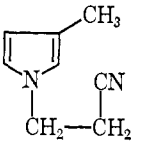
5

natural ketone, a free pair of adjacent α and β protons is required, placing the lone methyl group at C_7 as shown in formula **5**.

Synthetic confirmation of this assignment was obtained as follows. Cyanoethylation of 3-methylpyrrole (**6**) using Triton B as base catalyst gave the expected N-cyanoethyl-3-methylpyrrole (**7**) in 60% yield. The procedure for acid-catalyzed cyclization of **7** was based on that previously described for the cycli-



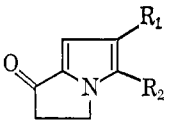
6



7

zation of N-cyanoethylpyrrole[12] and involved treatment of **7** with hydrogen chloride in dry ether. Hydrolysis of the resultant imine hydrochloride gave a ketone, mp 72–74°, identical with the natural ketone on the basis of mixture melting point and superimposable infrared, ultraviolet, nmr, and mass spectra. Rather curiously, the synthetic **5** lacked the characteristic sweet odor of the natural ketone. This odor must now be attributed to a trace impurity in the natural material. The possibility that the aromatic component may be of biological significance cannot be overlooked, and this point will be examined if suitable bioassays can be developed.

It must be pointed out that in a formal sense the cyclization of **7** might be expected to give either **5** or **8A**. In fact, a mixture of both isomers was antici-



8A, $R_1 = CH_3, R_2 = H$
B, $R_1 = H, R_2 = CH_3$

pated, but no evidence could be found for the formation of more than one 2,3-dihydromethyl-1H-pyrrolizin-1-one. Even though the cyclization yield was far from quantitative, it is hard to see why one isomer should be specifically lost in the work-up. We conclude that the 3-methyl group of **7** selectively activates C_2 for the intramolecular electrophilic substitution to a sufficient extent to bring about preferential formation of **5**. As stated earlier, the spin–spin coupling of the two aromatic protons in the product (J = 2.5 cps) supports this assignment, since the expected coupling of the two aromatic protons of **8A** would be much smaller (J = 1.5 cps).[13]

In an analogous fashion, 2-methylpyrrole was cyanoethylated and the resultant product cyclized and hydrolyzed to give the isomeric 2,3-dihydro-5-methyl-1H-pyrrolizidin-1-one (**8B**) which was clearly different from the natural heterocycle.

Ester I. The ester of shorter glpc retention time, designated originally as ester I, showed characteristic acetate absorption at 8.10 μ. The ester was unchanged when subjected to catalytic hydrogenation. In its mass spectrum, it showed a base peak at *m/e* 43 (CH_3CO^+). The molecular ion does not appear, but the highest *m/e* peak at 224, corresponding to a loss of acetic acid from a 284 parent, establishes the molecular weight. These data suggested the formula $C_{16}H_{33}OCOCH_3$, and a direct glpc comparison of ester I with an authentic sample of independently prepared *n*-hexadecyl (cetyl) acetate (**9**) showed identical retention times. Mass spectral comparison supported this structural assignment although a small amount of impurity with a molecular weight of 282 (calculated on the basis of an *m/e* 222 peak) could be clearly discerned. This suggests the presence of an accompanying unsaturated C_{16} acetate which was not further investigated.

$CH_3(CH_2)_{14}CH_2OCOCH_3$ **9**

$CH_3(CH_2)_{16}CH_2OCOCH_3$ **10**

Ester II. The ester of longer retention time, designated as ester II, also showed infrared and mass spectral characteristics of an acetate. The mass spectral molecular weight was 310, corresponding to the acetate of an unsaturated or monocyclic C_{18} alcohol. The first of these two possibilities was supported by the results of a catalytic hydrogenation experiment in which treatment of ester II in ethyl acetate solution with hydrogen and Adams catalyst at atmospheric pressure resulted in the formation of a *new* ester (II′), indistinguishable from an independently prepared sample of *n*-octadecyl (stearyl) acetate (**10**). The double bond in ester II appeared to be *cis*, since in the infrared it lacked the characteristic 10.25 μ absorption of *trans*-disubstituted double bonds. In fact, the infrared spectrum, mass spectrum, and glpc behavior of ester II were very similar to those of an authentic sample of oleyl acetate (**11**). However, since these properties

$CH_3(CH_2)_7-\underset{H}{C}=\underset{H}{C}-(CH_2)_7CH_2OCOCH_3$ **11**

$CH_3(CH_2)_7CO_2CH_3$ **12**

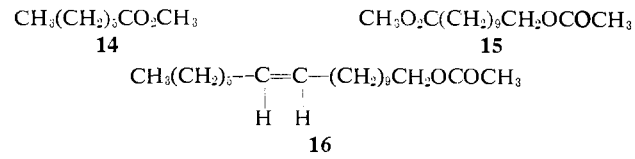
$CH_3O_2C(CH_2)_7CH_2OCOCH_3$ **13**

would not be expected to be sensitive to the position of the double bond, oxidative cleavage was used to establish this point. The periodate–permanganate technique of Lemieux and von Rudloff[14] is especially convenient on the milligram scale, and the technique was standardized using a 4-mg sample of oleyl acetate (**11**). The acidic products obtained from **11** were esterified with diazomethane. The more volatile fragment was indistinguishable from an authentic sample of methyl nonanoate (**12**), and the less volatile product was assumed to be the expected methyl 9-acetoxynonanoate (**13**).

The oxidative cleavage and subsequent esterification of 3.4 mg of ester II under conditions identical with those employed in the model study using **11** gave as

(14) R. U. Lemieux and E. von Rudloff, *Can. J. Chem.*, **33**, 1701 (1955); see also ref 15.

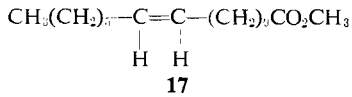
the more volatile product an ester identified as methyl heptanoate (**14**) by glpc comparison with an authentic



14 **15**

16

sample. This fixes the double bond of ester II between C_{11} and C_{12}. The higher molecular weight fragment had, as expected, a longer retention time than did the acetoxy ester **13**, and can be assigned structure **15** (methyl 11-acetoxyundecanoate). Ester II must therefore be *cis*-octadec-11-enyl (*cis*-vaccenyl) acetate (**16**). Reduction of an authentic sample of methyl *cis*-vaccenate (**17**)[15] with lithium aluminum hydride, followed by acetylation, gave a synthetic sample of **16** which proved indistinguishable from the natural product upon glpc comparison. Permanganate–periodate oxidation of **17**, followed by esterification, gave

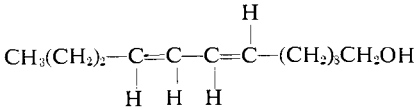


17

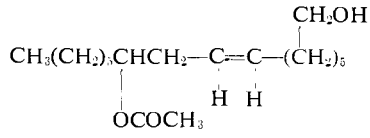
14 and **15**, identical with the fragments obtained previously from ester II.

Discussion

It is of interest to compare the structures of the major components of the *Lycorea* hairpencil secretion with those of some well-characterized pheromones (*i.e.*, compounds known to play a role in intraspecific communication).[3] In fact, very few pheromone structures have been established to date. It is striking that two of the best characterized ones are long-chain fatty alcohol derivatives. Thus, the sex attractant of the virgin female silk moth, *Bombyx mori*, has been shown to be hexadeca-*trans*-10,*cis*-12-dien-1-ol (**18**),[16] while



18



19

the sex lure of the gypsy moth, *Porthetria dispar*, has been characterized as (+)-10-acetoxyhexadec-*cis*-7-en-1-ol (**19**).[17] The superficial resemblance of these structures (**18** and **19**) to those of the hairpencil esters (**9** and **16**) is at once apparent, although given that the biological role of these compounds must be entirely different[18] the meaning of this similarity remains to be explained.

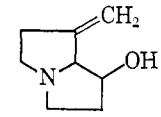
(15) This ester was obtained from the Hormel Institute, University of Minnesota. We are most grateful to Drs. K. Bloch and J. Law for calling this source to our attention.

(16) A. Butenandt, R. Beckmann, D. Stamm, and E. Hecker, *Z. Naturforsch.*, **146**, 283 (1959).

(17) M. Jacobson, M. Beroza, and W. A. Jones, *J. Am. Chem. Soc.*, **83**, 4819 (1961).

(18) These female moth secretions are concerned with attracting males at a very great distance, while the butterfly's use of the hairpencils seems to be concerned with inducing an already nearby female to stop flying and prepared for mating.

The heterocyclic ketone **5** bears no close resemblance to any previously characterized natural product from the animal kingdom, but is strikingly similar to the Senecio and Crotalaria alkaloids.[19] A typical member of this family is **20**, which shows not only the same basic



20

nucleus, but also has a single carbon substituent at C_7 and an oxygen substituent at C_1.[20] While none of these alkaloids seems to possess an aromatized pyrrole ring, this is a relatively small structural deviation. Given the close relationship of **5** to a known family of plant alkaloids, it is possible that **5** may not be produced by the butterfly itself, but may be obtained or derived from a food source. We hope it will be possible to investigate this question in the future. However, the prime requirement at this point is to see whether pheromonal activity can be demonstrated in the natural and synthetic hairpencil components. Future research in this area will depend on the results of such studies.

Experimental Section

Melting points were determined on a Thomas–Hoover capillary melting point apparatus and are uncorrected. The infrared spectra were recorded on a Perkin-Elmer Infracord. The ultraviolet measurements were made on a Cary Model 14 recording spectrophotometer. The nmr spectra were determined with a Varian Associates A-60 spectrometer. Glpc analyses were performed on an Aerograph Hy-Fl Model 600 and Model 660. Columns of 6 ft × ⅛ in. were used. Mass spectra were determined by Drs. A. F. Thomas and B. Willhalm, Firmenich & Cie, Genève, Switzerland, using an Atlas CH-4 mass spectrometer. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tennessee.

Isolation of Organic Components from Hairpencils. The hairpencils from 100 live male *Lycorea ceres* were pulled off individually and soaked in 5 ml of Spectrograde methylene chloride or carbon disulfide. After a few hours, the solvent was taken up into a capillary dropper and filtered. The solution was concentrated to about 1 ml and its infrared spectrum recorded directly. After four such extractions, no further extractable material was obtained. The combined extract showed strong infrared absorption (CS_2) at 3.45, 3.52, 5.77, 5.92, and 8.10 μ. Glpc analysis at 180° (5% SE 30 column) showed three major components at 1.7, 10.5, and 20 min.

Separation of Components. (1) The combined hairpencil extract from 100 butterflies was concentrated to a small volume and chromatographed on 15 g of silicic acid[21] and eluted with methylene chloride–methanol. Upon elution with pure methylene chloride, 28 mg of ester mixture was obtained as a clear oil. The infrared spectrum of this fraction in CS_2 showed strong absorption at 5.77 μ in the carbonyl region, with other maxima at 3.42, 3.52, and 8.10 μ. Glpc analysis (5% SE 30 column) showed the presence of two main components. Further separation of the two esters by column chromatography was unsuccessful. The esters migrated as one spot upon thin layer chromatography using silica gel. Their separation *via* preparative glpc is described below.

With 1% methanol in methylene chloride, about 10 mg of a ketonic fraction was obtained from the chromatogram as a viscous oil. This fraction solidified upon standing in the refrigerator overnight and showed mp 63–66°. It was further purified by sublimation at 60–65° (0.5 mm) to give 2.3 mg of colorless needles, mp 74–75° (softens at 65°). Glpc analysis on a 5% SE 30 column

(19) See N. J. Leonard in "The Alkaloids," Vol. 1, R. H. F. Manske and H. L. Holmes, Ed., Academic Press Inc., New York, N. Y., 1950, p 108; Vol. 6, 1960, p 37.

(20) C. C. J. Culvenor, L. W. Smith, and W. G. Woods, *Tetrahedron Letters*, 2025 (1965).

(21) Mallinkrodt silicic acid, acid washed and fines removed, dried at 150° for 16 hr.

showed only a single fraction, at 1.7 min (180°); infrared spectra: (in CH_2Cl_2) 3.4 (w), 5.95 (s), 6.45 (m), 6.80 (w), 7.20 (s), 7.58 (m), 7.70 (w), 8.20 (w), 9.32 (m), 9.65 (m), and 9.90 μ (w); (in CS_2) 3.40 (w), 5.92 (s), 7.20 (s), 7.58 (m), 7.70 (m), 8.20 (w), 9.32 (m), 9.65 (m), 9.90 (w), and 13.4 μ (s); ultraviolet spectrum: λ_{max}^{EtOH} 288 mμ (log ϵ 4.22); mass spectrum: parent peak 135, base peak 135; other fragments (decreasing intensity): 107, 79, 52, 27, 39, 65, 92, 120 (*m/e*).

(2) The crude extract from 200 hairpencils (obtained from 100 live butterflies) was subjected directly to sublimation at 60–65° (0.5–1.0 mm) for 1–2 hr. The ketonic component (4.5 mg) was obtained as a crystalline solid, mp 74–75° (softens at 66°); nmr (in CCl_4 with internal tetramethylsilane) τ 7.80 (3 H, singlet), 7.16 (2 H, triplet, J = 6.5 cps), 5.87 (2 H, triplet, J = 6.5 cps), 3.91 (1 H, doublet, 2.5 cps), 3.31 (1 H, doublet, 2.5 cps).

The residue was subjected to preparative glpc at 200° on a 5% SE 30 column, using a stream splitter, to give 4.5 mg of ester I (5 min) and 8.5 mg of ester II (8.5 min); mass spectra: ester I base peak 43; M − 60; *m/e* 224 (this spectrum matched that of authentic *n*-hexadecyl acetate, but showed additional intensities at 222 and in general at 2 mass units below the various *n*-hexadecyl acetate fragments); ester II base peak 43; M − 60; *m/e* 250.

Hydrogenation of the Ester Mixture. The ester mixture (~20 mg) was dissolved in 200 μl of ether; 20 μl of the solution was transferred into a preweighed microhydrogenation flask. The ether was evaporated in a stream of dry nitrogen. The residue weighed 1.8 mg. This residue was hydrogenated at room temperature and atmospheric pressure in 0.5 ml of ethyl acetate with a small amount of platinum dioxide catalyst for 3 hr. At the end of this period the catalyst was removed by filtration and the solvent by evaporation under a stream of nitrogen. The residue (1.9 mg) was taken up in 100 μl of ether and used directly for glpc comparisons on the 5% SE 30 column. The original ester mixture showed ester I at 10.5 min (180°) and ester II at 20 min (180°). The hydrogenated ester mixture again showed ester I at 10.5 min (180°) and a new ester, II′, at 21.2 min (180°). A mixture of ester I and 1-hexadecyl acetate (**9**) showed only a single peak at 10.5 min (180°). A mixture of the hydrogenated ester II′ and 1-octadecyl acetate (**10**) also showed only a single peak at 21.5 min (180°). Finally, a mixture of ester II and oleyl acetate (**11**) showed a single peak at 20 min (180°).

Periodate–Permanganate Oxidation of Oleyl Acetate.[14] A stock solution was prepared by dissolving 224 mg of potassium periodate and 12 mg of potassium permanganate in 50 ml of distilled water. A mixture of 4 mg of oleyl acetate,[22] 1.8 ml of *t*-butyl alcohol, and 3 ml of the oxidation stock solution was brought to pH 8–9 by dropwise addition of aqueous potassium carbonate. The reaction mixture was stirred for 20 hr with a magnetic stirrer. At the end of this period, the mixture was extracted with ether to remove all the neutral material. The aqueous layer was then acidified with hydrochloric acid, and a few drops of sodium bisulfite solution was added to decolorize the reaction mixture. The acidic products were extracted with methylene chloride and dried over magnesium sulfate. After removal of the solvent, the residue was evaporatively distilled at 60–70° (0.1 mm) (bath temperature) to give 2.6 mg of distillate. The infrared spectrum of this fraction showed both acetate and carboxylic acid absorption at 5.77 and 5.85 μ in CS_2 solution.

These acids were dissolved in ether and treated with an ethereal solution of diazomethane. The resulting methyl esters were used for direct comparison by glpc on the 5% SE 30 column. A peak at 5.7 min (115°) corresponded to methyl nonanoate (**12**). Under identical conditions methyl octanoate appeared at 3.5 min (115°) and methyl decanoate at 9.7 min (115°). At 155° the esterified oxidation product showed the peaks corresponding to methyl nonanoate (**12**) at 2 min and methyl 9-acetoxynonanoate (**13**) at 10 min.

Periodate–Permanganate Oxidation of Ester II. Ester II (3.4 mg) was oxidized in 1.2 ml of *t*-butyl alcohol, using 3 ml of oxidation stock solution under conditions identical with those described for the oxidation of oleyl acetate. The acidic product showed an infrared spectrum in CS_2 solution indistinguishable from that derived from oleyl acetate. This acidic fraction was dissolved in ether and treated with an ethereal solution of diazomethane. Glpc analysis (5% SE 30 column) showed a new methyl ester indistinguishable from methyl heptanoate, at 2.6 min (110°). At higher temperature a second component at 8.6 min (175°) was observed. Under comparable conditions methyl 9-acetoxynonanoate (**13**) from oleyl acetate appeared at 4.3 min (175°).

Periodate–Permanganate Oxidation of *cis*-Vaccenyl Acetate. Methyl *cis*-vaccenate (**17**) (130 mg) was added dropwise to a slurry of lithium aluminum hydride (79 mg) in 5 ml of anhydrous ether under a nitrogen atmosphere. The reaction mixture was stirred at room temperature overnight. The excess hydride was decomposed by slow addition of water. The inorganic solid was removed by filtration and washed with ether. The combined filtrate was dried with magnesium sulfate. After evaporation of the solvent, the alcohol weighed 123 mg and showed absorption in the infrared at 2.95 μ (neat). The crude alcohol was dissolved in 1 ml of dry pyridine and treated with 50 capillary drops (about 0.5 ml) of acetic anhydride. The reaction mixture was stoppered and left at room temperature for 24 hr. A few drops of water were added to decompose the excess acetic anhydride. When the reaction subsided, an additional 3 ml of water was added. The product was extracted with methylene chloride, washed with dilute hydrochloric acid and 10% sodium carbonate, and dried over magnesium sulfate. Upon removal of solvent the crude acetate (**16**) weighed 146 mg. This product was distilled at 128–140° (0.2 mm) (bath temperature) to yield 115 mg of **16**, showing characteristic carbonyl absorption at 5.77 μ (CS_2).

Glpc analysis (5% SE 30 column) showed a single peak at 8.5 min (200°). A mixture of *cis*-vaccenyl acetate and ester II from *Lycorea ceres* showed a single gas chromatographic peak.

Synthetic *cis*-vaccenyl acetate (**16**) (4 mg) was oxidized under exactly the same conditions as were described for oleyl acetate. The acidic fraction was esterified with diazomethane. Glpc comparison on the 5% SE 30 column showed both fragments to be identical in retention times with those obtained from ester II.

β-Methylpyrrole (6). This pyrrole was prepared from N-acetylphthalimide and the sodium salt of diethyl oxalacetate in four steps, by the method of Lancaster and Van der Werf.[23]

Cyanoethylation of β-Methylpyrrole. The technique for the cyanoethylation of pyrrole[24] was adapted for the cyanoethylation of **6**. A mixture of 1.8 g (0.022 mole) of β-methylpyrrole (**6**) and 0.2 ml of Triton B (40%) was stirred under a nitrogen atmosphere. Acrylonitrile (1.6 ml, 1.28 g, 0.024 mole) was added dropwise over 15 min. The reaction temperature was maintained below 40° with occasional cooling by means of an ice–water bath. After stirring at room temperature overnight the reaction mixture was diluted with ether, washed with water and saturated sodium chloride solution, and dried over magnesium sulfate. After removal of solvent the nitrile was distilled at 88–90° (0.3 mm) to give 1.85 g (60% yield) of **7**; infrared spectrum: 4.45 μ (neat).

Anal. Calcd for $C_8H_{10}N_2$: C, 71.61; H, 7.51; N, 20.88. Found: C, 71.40; H, 7.72; N, 21.12.

2,3-Dihydro-7-methyl-1H-pyrrolizidin-1-one (5). The cyclization of **7** was patterned after the procedure of Josey and Jenner.[12] The nitrile **7** (1.8 g, 0.0134 mole) was dissolved in 10 ml of anhydrous ether, and the solution was cooled with an ice–salt bath. A dry stream of hydrogen chloride was bubbled in slowly for 2 hr. During that period, orange crystals of the imine hydrochloride deposited. The reaction mixture was stoppered and refrigerated overnight. The ether was decanted and the orange solid washed once with cold ether. It was then dissolved in 15 ml of water, along with 4 g of sodium acetate. Benzene (10 ml) was added. The reaction mixture was stirred at 90° for 1 hr with a reflux condenser. The benzene layer was separated, and the aqueous layer was washed with another portion of benzene, rehydrolyzed in the same manner for another hour, and reextracted. The combined benzene extract was washed with 10% sodium carbonate and dried. Upon evaporation of the solvent, the crude ketone appeared as a brown oily residue. Sublimation at 70° (0.3 mm) gave 0.55 g (30% yield) of a colorless solid (**5**), mp 72–74° (softens at 65°). A mixture melting point with the ketonic component from *Lycorea ceres* was undepressed. A small sample was resublimed for elemental analysis.

Anal. Calcd for C_8H_9NO: C, 71.09; H, 6.71; N, 10.36. Found: C, 70.77; H, 6.74; N, 10.45. The infrared, ultraviolet, nmr, and mass spectra of the synthetic material were identical with that of the natural product.

α-Methylpyrrole. This pyrrole was prepared by the lithium aluminum hydride reduction of α-pyrrole carboxaldehyde according to the procedure of Hinman and Theodoropulos.[25]

(22) Prepared by acetylation of oleyl alcohol supplied by the Applied Science Laboratory, State College, Pa.

(23) R. E. Lancaster, Jr., and C. A. Van der Werf, *J. Org. Chem.*, **23**, 1208 (1958).

(24) R. C. Blume and H. G. Lindwall, *ibid.*, **10**, 255 (1945).

(25) R. L. Hinman and S. Theodoropulos, *ibid.*, **28**, 3052 (1963).

Cyanoethylation of α-Methylpyrrole. α-Methylpyrrole (2 g) was cyanoethylated as described above for the β-methyl isomer. The product was distilled at 84° (0.25 mm) to give 2.15 g of the expected nitrile **8B**; infrared spectrum: 4.45 μ (neat).

Anal. Calcd for $C_8H_{10}N_2$: C, 71.61; H, 7.51; N, 20.80. Found: C, 71.46; H, 7.66; N, 20.90.

2,3-Dihydro-5-methyl-1H-pyrrolizidin-1-one (8B). The cyclizations of 1 g of the above nitrile was carried out as described previously for the preparation of **5**. Upon sublimation of the crude product only a liquid film was obtained on the cold finger. The infrared spectrum showed absorption at 5.92 and 4.45 μ, which showed incomplete cyclization. Glpc analysis on a 10% Carbowax column (225°) showed the new ketone (**8B**) at 3 min, as compared with **5** at 6 min. The cyclization was not further studied since the ketone thus obtained was clearly different from the natural product.

Simultaneous Synthesis of 1-Hemi-D-cystine-oxytocin and Oxytocin and Separation of the Diastereoisomers by Partition Chromatography on Sephadex and by Countercurrent Distribution[1]

Donald Yamashiro, Dieter Gillessen, and Vincent du Vigneaud

Contribution from the Department of Biochemistry, Cornell University Medical College, New York, New York 10021. *Received December 4, 1965*

Abstract: Oxytocin and 1-hemi-D-cystine-oxytocin have been synthesized simultaneously and separated by partition chromatography on Sephadex and by countercurrent distribution. *p*-Nitrophenyl N-carbobenzoxy-S-benzyl-DL-cysteinate was coupled with L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide, and the resulting mixture of diastereoisomeric protected polypeptide amides was treated with sodium in liquid ammonia and then oxidized to yield a mixture of oxytocin and 1-hemi-D-cystine-oxytocin. The latter diastereoisomers were effectively separtated by partition chromatography on Sephadex G-25 in the solvent system 1-butanol–benzene–pyridine–0.1% acetic acid (6:2:1:9) and by countercurrent distribution in the same solvent system. Both procedures yielded oxytocin with a potency comparable to that previously reported for the highly purified hormone. The 1-hemi-D-cystine-oxytocin obtained by either method possessed an oxytocic potency of about 2 units/mg, which remained constant after submission of the compound to further chromatography.

In previous syntheses of 1-hemi-D-cystine-oxytocin,[2,3] S-benzyl-D-cysteine has been used as the starting material for the introduction of the hemi-D-cystine residue in the 1 position of oxytocin (Figure 1). In starting with S-benzyl-D-cysteine, if any traces of the L isomer are present or if any slight racemization should take place in the subsequent synthetic steps, oxytocin itself becomes a contaminant.

In preliminary experiments it was found that oxtocin could be cleanly separated from 1-hemi-D-cystine-oxytocin by partition chromatography on Sephadex in the solvent system 1-butanol–benzene–pyridine–0.1% acetic acid (6:2:1:9). It therefore occurred to us that by starting with S-benzyl-DL-cysteine one could prepare simultaneously both diastereoisomers and then separate one from the other. Thus one would obtain highly purified preparations of both oxytocin and 1-hemi-D-cystine-oxytocin. This has been accomplished and is being presented in this communication. This isolation of highly purified 1-hemi-D-cystine-oxytocin has also afforded a further examination of the pharmacological activities of this analog.

Previous efforts to separate oxytocin from 1-hemi-D-cystine-oxytocin by countercurrent distribution in the solvent system 1-butanol–1-propanol–0.05% acetic acid (3:2:5) at 4° were unsatisfactory.[3] The question arose as to whether this lack of separation was due to the nature of the solvent system that had been employed. We therefore thought it would be of interest to see whether these two diasteroisomers could be separated by countercurrent distribution in the solvent system that had been used on Sephadex. Such a separation did occur upon countercurrent distribution in the 1-butanol–benzene–pyridine–0.1% acetic acid (6:2:1:9) solvent system.

For synthesis the stepwise *p*-nitrophenyl ester method[4] was used, the last step being the coupling of *p*-nitrophenyl S-benzyl-N-carbobenzoxy-DL-cysteinate with the octapeptide L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide. The resulting mixture of diastereoisomeric protected nonapeptides was treated with sodium in liquid ammonia for removal of the benzyl and carbobenzoxy groups according to the method of Sifferd and du Vigneaud.[5] The resulting disulfhydryl nonapeptides were oxidized to the cyclic octapeptides by the method of Weygand and Zumach[6] with the use of 1,2-diiodoethane in aqueous acetone, the disappearance of sulfhydryl groups being followed quantitatively by the method of Ellman.[7] It has recently been found in

(1) This work was supported in part by Grant HE-01675 from the National Heart Institute, U. S. Public Health Service. Dr. Gillessen wishes to acknowledge a Fulbright Travel Grant.

(2) K. Jošt, J. Rudinger, and F. Šorm, *Collection Czech. Chem. Commun.*, **26**, 2496 (1961); **28**, 2021 (1963).

(3) D. B. Hope, V. V. S. Murti, and V. du Vigneaud, *J. Am. Chem. Soc.*, **85**, 3686 (1963).

(4) M. Bodanszky and V. du Vigneaud, *Nature*, **183**, 1324 (1959); *J. Am. Chem. Soc.*, **81**, 5688 (1959).

(5) R. H. Sifferd and V. du Vigneaud, *J. Biol. Chem.*, **108**, 753 (1935).

(6) F. Weygand and G. Zumach, *Z. Naturforsch.*, **17b**, 807 (1962).

(7) G. L. Ellman, *Arch. Biochem. Biophys.*, **82**, 70 (1959).