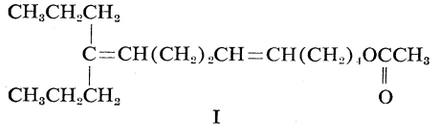
methylsilane as a reference. The spectra gave evidence (chemical shift in values of δ) for three olefinic protons (δ, 5.40), two methylene protons adjacent to an acetate oxygen atom (δ, 4.06), ten methylene protons adjacent to double-bonded carbon atoms (δ, 2.03), three acetyl methyl protons (δ, 2.03), eight methylene protons adjacent to either methylene or methyl groups (δ, 1.34), and six terminal methyl protons (δ, 0.89) that appeared to be separated from a double-bonded carbon by at least two methylene groups. Double resonance studies indicated at least three methylene groups between the acetate group and a double bond, and only two methylene groups between the double bonds.

The only structure for the attractant consistent with the foregoing data is 10-propyl-*trans*-5, 9-tridecadienyl acetate (I), and this structure was confirmed by an 11-step synthesis.



I

Compound I was synthesized in 0.2-percent overall yield by the following procedure. Condensation of 4-heptanone with ethyl bromoacetate in the presence of zinc (*14*) gave ethyl 3-hydroxy-3-propylcaproate (75 percent; bp, 85°C at 1.5 mm-Hg), which was dehydrated with phosphorus oxychloride in pyridine to a mixture (88 percent; bp, 105° to 109°C at 20 mm; n_D^{25}, 1.4412) consisting of approximately 50 percent of ethyl 3-propyl-2-hexenoate and 50 percent of ethyl 3-propyl-3-hexenoate. Distillation through a spinning band column gave the pure α,β-isomer (bp, 116.5°C at 28 mm) and the pure β,γ-isomer (bp, 113.5°C at 28 mm) as colorless liquids. Ethyl 3-propyl-2-hexenoate was reduced with lithium aluminum hydride to 3-propyl-2-hexen-1-ol (*15*) (80 percent; bp, 125°C at 25 mm; $n_D^{23.5}$, 1.4520), which was converted with phosphorus tribromide in pentane to 1-bromo-3-propyl-2-hexene (72 percent; bp, 105°C at 40 mm and 63°C at 3.3 mm; n_D^{27}, 1.4805). Stirring this compound for 6 days at room temperature with sodium cyanide in ethanol and hydrolysis of the crude product with alcoholic alkali gave crude 4-propyl-3-heptenoic acid, which was reduced with lithium aluminum hydride to 4-propyl-3-hepten-1-ol (*15*) (bp, 108° to 112°C at 15 mm); overall yield from the bromide was 76 percent. Treatment of this olefinic alcohol with phosphorus tribromide gave the unstable 1-bromo-4-propyl-3-heptene (76 percent; bp, 82° to 83°C at 2.5 mm; $n_D^{21.5}$, 1.4716), which was coupled, without delay, with the tetrahydropyranyl ether of 5-hexyn-1-ol (*16*) by means of sodamide in liquid ammonia. The resulting 10-propyl-1-(tetrahydro-2-pyranyloxy)-tridec-9-en-5-yne (23 percent; bp, 175°C at 0.5 mm; n_D^{27}, 1.4729) was reduced with sodium in liquid ammonia to 10-propyl-1-(tetrahydro-2-pyranyloxy)-*trans*-5,9-tridecadiene (85 percent; bp, 135°C at 0.1 mm; n_D^{21}, 1.4721), which was then hydrolyzed at room temperature with methanolic sulfuric acid to 10-propyl-*trans*-5, 9-tridecadien-1-ol (90 percent; bp, 110° to 120°C at 0.08 mm-Hg; n_D^{25}, 1.4715). Refluxing this alcohol with acetyl chloride in anhydrous benzene gave compound I (16 percent) as a colorless liquid (bp, 135°C at 0.1 mm; n_D^{25}, 1.4635) identical in all respects with the natural attractant.

The sex attractant, for which the name "propylure" is suggested, is believed to be the first-reported natural constituent possessing propyl branching.

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8. Mesh 60-100, obtained from the Floridin Co., Tallahassee, Fla., and treated to contain 3 percent water. The mention of trade names or products does not constitute endorsement by the U.S. Department of Agriculture over those not named.
9. The hexane used in these investigations was purified to the equivalent of spectral grade by percolation of reagent-grade hexane through silica gel and distillation. The ethyl ether was distilled and stored over sodium. All other solvents used were reagent grade, unless otherwise specified.
10. Adsorbosil-CABN, 60-100 mesh, containing 10 percent calcium sulfate binder and 25 percent silver nitrate, obtained from Applied Science Laboratories, State College, Pennsylvania.
11. The chromatography was carried out on an Aerography "Autoprep" instrument, model 700, obtained from Varian Aerograph, Walnut Creek, California, with a stainless steel column (2.4 m by 0.63 cm diameter), packed with 5 percent SE-30 on Chromosorb W; column temperature, 185°C; helium flow rate, 33.3 ml/min.
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15. Additional amounts of this compound, prepared by a different method, were later obtained from Midwest Research Institute, Kansas City, Missouri.
16. 5-Hexyn-1-ol, prepared in 75-percent yield by the method of G. Eglinton, E. R. H. Jones and M. C. Whiting, *J. Chem. Soc.* **1952**, 2873 (1952), or purchased from Farchan Research Laboratories, Willoughby, Ohio, was converted to its tetrahydropyranyl ether (bp, 70° to 80°C at 0.3 mm; n_D^{25}, 1.4556) in 76-percent yield.
17. We thank Drs. M. T. Ouye and M. J. Lukefahr and Mr. J. M. McGough, all of the U.S. Dept. of Agriculture, Brownsville, Texas, for supplying the large numbers of insects necessary for the isolation and laboratory bioassay investigations. We thank Dr. H. Fales, NIH, Bethesda, Maryland, for running the mass spectrum; E. Pier and Drs. L. F. Johnson and N. Bhacca, all of Varian Associates, Palo Alto, California, for obtaining and interpreting the nuclear magnetic resonance spectra; and R. Sarmiento, USDA, Beltsville, Maryland, for the hydrogenolytic gas-chromatographic determination.

19 May 1966

Allelic Antigenic Factor Inv(a) of the Light Chains of Human Immunoglobulins: Chemical Basis

Abstract. *Twenty-seven Bence Jones proteins of immunological type K show a common set of peptides. One of the common peptides differs in three of the proteins which are the only ones classified by a serological test as Iav(a+). The difference in the peptide analyzed is caused by a valine-leucine interchange; Inv(a+) proteins have leucine, whereas Inv(a−) proteins have valine in position 189.*

Genetic variants (designated Gm and Inv) of the peptide chains of human immunoglobulins having distinct serological properties have been described (*1*). The Gm factors are present in heavy chains of γG immunoglobulins (*2*), whereas the Inv factor is present in light chains (*2*). The Bence Jones proteins, which correspond to the light chains of immunoglobulins (*3*), offer a

unique opportunity for the study of the chemical basis of the Inv factor. The Bence Jones protein excreted by an individual with myeloma is in most cases a homogeneous protein which differs from Bence Jones proteins excreted by other patients with myeloma (*4*). Each individual Bence Jones protein is of either type K or L as distinguished by immunological methods; the type K Bence Jones protein is either Inv(a+) or Inv(a−) (*5*).

In a systematic study of the chemical structure of the human immunoglobulins we have examined a group of 27 type K Bence Jones proteins. These proteins were aminoethylated and purified by gel filtration on Sephadex G-100, and the purified proteins were then lyophilized and digested with trypsin (*6*). The tryptic digests were then separated by two-dimensional electrophoresis and chromatography (fingerprint) in order to study the heterogeneity of Bence Jones proteins (*7*). A pattern of peptides common to all type K Bence Jones proteins was observed in fingerprints (*8*), in agreement with the similar finding of Putnam and Easley (*4*).

These "common" peptides have been isolated (*7*), and by their amino acid composition have been identified with peptides belonging to the COOH-terminal half of the amino acid sequence of type K Bence Jones proteins (*9, 10*). They occupied an identical position in fingerprints of type K Bence Jones proteins and gave identical reactions with reagents specific for given amino acids (*6*). The one exception was the peptide designated 9A (Fig. 1), which showed, upon chromatography, a higher R_F in the fingerprints of some Bence Jones proteins (Fig. 1*B*).

The Bence Jones proteins were classified for the Inv(a) antigenic specificity (*11*); three proteins were Inv(a+). Fingerprints of Inv(a−) proteins differed from fingerprints of Inv(a+) proteins in the position in chromatography of peptide κ9A. This peptide gave a positive reaction with the α-nitroso-β-naphthol (a test for tyrosine) and with the platinic iodide reagent for reduced sulfur (*6*) in both groups of peptide patterns. The other "common" peptides were identical in the two groups of Bence Jones proteins; differences in the peptide maps were limited to the "distinctive" peptides. Thus it seemed likely that the amino acid composition of peptide κ9A from Inv(a−) Bence Jones proteins differs from that of κ9A from Inv(a+) proteins. This difference may account for the antigenic behavior of these two groups of proteins in the Inv(a) serological test.

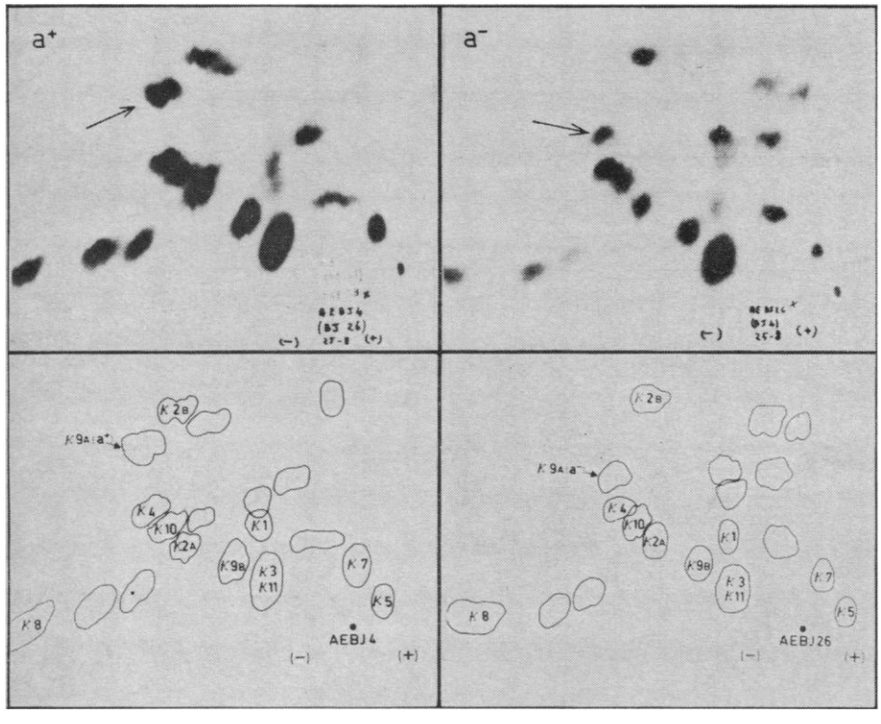

Fig. 1. Photographs (top) and tracings (bottom) of the peptide patterns of tryptic digests of the aminoethylated Bence Jones proteins BJ 26(a−) (right) and BJ 4(a+) (left). After aminoethylation the proteins were purified and digested with trypsin (*6*); 2.5 mg of tryptic digest were separated by electrophoresis at *p*H 6.4 and by chromatography in a mixture of isoamyl alcohol, pyridine, and water (35:35:30) (*6*). The arrows indicate peptides κ9A(a+) and κ9A(a−); these peptides have a different R_F in chromatography. The "common" peptides are indicated in the tracings by the Greek letter κ and a number, which indicates their position along the amino acid sequence of the "common" part of type K peptide chains of immunoglobulins (*9*). Peptides indicated by the same numeral followed by the letter *A* or *B* are peptides obtained by trypsin cleavage at the position where aminoethylcysteine occurs in the chain; thus peptides designated *A* have aminoethylcysteine as the COOH-terminal amino acid. Not all tryptic peptides appear in these peptide patterns; a few peptides are presumably removed with the insoluble material that is found at the end of the tryptic digestion (*6*).

Peptide κ9A was purified from the tryptic digest of an Inv(a+) and of an Inv(a−) protein; these peptides will be designated κ9A(a+) and κ9A(a−), respectively. The tryptic digest of protein BJ 4(a+) was fractionated by gel filtration on a column of Bio-Gel P-2 (Fig. 2). The fractions separated were pooled and concentrated; portions were analyzed by high-voltage electrophoresis on paper at *p*H 4.7 (*12*) to locate peptide κ9A(a+) with ninhydrin, α-nitroso-β-naphthol, and platinic iodide reagents. This peptide was found in peak 5. The peptides of protein BJ 26(a−) separated in a similar, although not identical, pattern; peptide κ9A(a−) was located in the same way in one peak of the chromatogram. The fractions, pooled from the Bio-Gel column for further study, each contained four or five peptides, which were separated by column chromatography on Dowex 50 X-2 (*13*). The peptide κ9A was located by high-voltage electrophoresis of the fractions corresponding to peaks of the chromatogram.

Peptides κ9A(a+) and κ9A(a−) were analyzed for their amino acid composition and for their NH_2-terminal amino acid as previously indicated (*12*). The peptides were digested with chymotrypsin, and the digestion products were separated by preparative high-voltage electrophoresis (*12*), two peptides resulting from each of the peptides analyzed. These chymotryptic peptides were located with ninhydrin on guide strips, eluted with 6*N* HCl from the electrophoresis paper, hydrolyzed, and analyzed (Table 1).

Table 1. Amino acid sequence of peptides κ9A(a −) and κ9A(a +). Amino acids valine, tyrosine, and leuceine are indicated by the first three letters of their names. Aminoethylcysteine is indicated by AECys; this amino acid is the COOH-terminal amino acid of peptides κ9A(a +) and κ9A(a −) because of the specificity of trypsin.

κ9A(a −)	κ9A(a +)
Amino acid composition	
(ValTyrAlaAECys)+	(LeuTyrAlaAECys)
NH_2-terminal amino acid	
Val	Leu
Products of chymotryptic hydrolysis	
(ValTyr)	(LeuTyr)
(AlaAECys)	(AlaAECys)
Amino acid sequence	
Val.Tyr.Ala.AECys	Leu.Tyr.Ala.AECys
Position in the sequence of κ-chain	
189.....192	189.....192

The amino acid sequence was established for each of the peptides κ9A(a+) and κ9A(a−). These peptides differ by one amino acid residue only, the NH_2-terminal of the peptide: leucine is the NH_2-terminus of peptide κ9A(a+) whereas valine is the NH_2-terminus of peptide κ9A(a−). The amino acid sequence established for the tetrapeptide κ9A(a+) allowed us to locate it in the sequence of the Bence Jones protein Roy (*9*); this protein is Inv(a+). The sequence established for the tetrapeptide κ9A(a−) allowed us to locate it in the sequence of the Bence Jones proteins Cu (*9*) and Ag (*10*); these proteins are Inv(a−). Both peptides κ9A(a+) and κ9A(a−) occupy positions 189 to 192 of the amino acid sequence, and the valine-leucine interchange occurs in position 189. The COOH-terminal half (from residue 107 to residue 212) of the amino acid sequence of type K Bence Jones proteins is identical in three Bence Jones proteins analyzed, except for the interchange in position 189 (*9*, *10*). This interchange has already been pointed out; Hilschmann and Craig (*9*) have observed it in proteins which were not of the same genetic type. By examining a large number of Bence Jones proteins we have now provided evidence that the presence of leucine or valine in position 189 is correlated with the Inv(a) antigenic type of the protein. Inv(a+) proteins have leucine, whereas Inv(a−) proteins have valine in position 189.

It seems possible that a single amino acid difference accounts for the distinct serological properties of other variants of immunoglobulins controlled by allelic genes. A single-spot difference has been observed between the "common" peptide patterns of Gm(a+) and of Gm(a−) human γG immunoglobulins (*14*). The possibility that these immunoglobulin molecules differ by a single amino acid in their common sequence has the following implications: (i) Allelic variants of immunoglobulins show changes of the type observed (for instance) in abnormal hemoglobins, that is, the substitution of a single amino acid residue. (ii) Such substitutions may help to recognize parts of these complex proteins which are controlled by "true" alleles (*15*). In fact immunoglobulins are probably controlled by chromosomal regions which cannot be easily separated into component structural genes on the basis of formal genetic analysis. (iii) A single amino acid substitution in a complex protein is sufficient for inducing a different antigenic specificity which can be recognized by antibody. This is particularly noteworthy in the case of the Inv serological system since it is not likely that the interchange of valine and leucine causes a drastic change in the tertiary structure of these proteins.

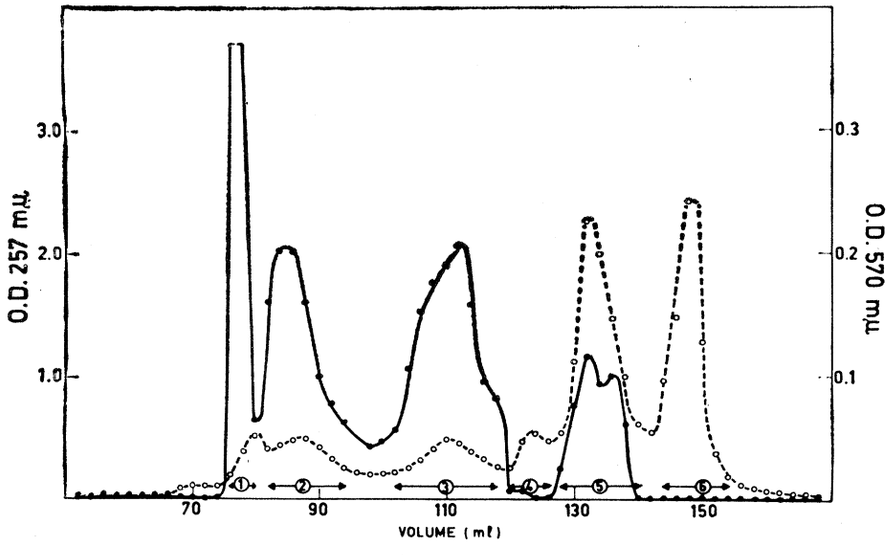

Fig. 2. Separation of the tryptic peptides of Bence Jones protein BJ 4 on Bio-Gel P-2 (100–150). The column (120 by 1.4 cm) was equilibrated with distilled water. The tryptic digest of 200 mg of aminoethylated protein was applied to the column and eluted with distilled water. Fractions (2 ml) were collected and analyzed for optical density at 257 mμ (broken line); 20 μl of each fraction was reacted with ninhydrin (*11*) and analyzed for optical density at 570 mμ (solid line). The fractions pooled for further study are indicated by arrows.

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