cleavage of the intermediate alkyliridium(III) complex with bromine had the same configuration as the starting **5**, and on the *assumption* that bromination of this alkyliridium complex occurred with *retention* of configuration at carbon.[13,14] The *inversion* of configuration established for bromination of **1** indicates that this proposal should presently be accepted with reservation. Although the stereochemistry assumed for the bromination leading to **5** may ultimately be demonstrated to be correct, it is clear that either inversion or retention of configuration may characterize brominative cleavage of carbon–metal bonds.

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(15) National Institutes of Health Predoctoral Fellow, 1967–1970.

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Amino Acid Sequence of the Subunits of Ovine Pituitary Interstitial Cell-Stimulating Hormone

Sir:

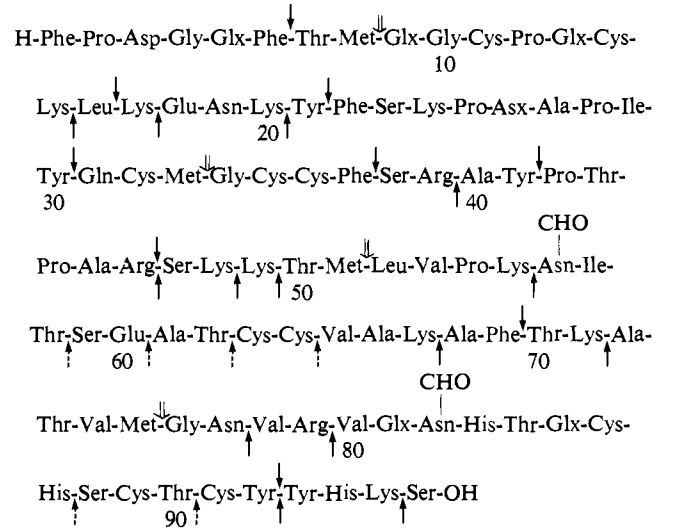
Ovine interstitial cell-stimulating hormone (ICSH, LH), a glycoprotein of about 30,000 molecular weight,[1,2] is a dimer[3] in neutral solutions. Carboxyl terminal group analysis by Ward, *et al.*,[4] suggested the presence of two nonidentical polypeptide chains. Subsequently, by virtue of their isolation by countercurrent distribution,[5] the subunits of ICSH were unequivocally demonstrated to be chemically dissimilar both with respect to amino acid and carbohydrate content. The individual subunits, ICSH-α and ICSH-β (formerly designated ICSH-CI and ICSH-CII), are biologically inactive until recombined.[6-8] Enzyme digestion with carboxypeptidase A showed[9] that the COOH terminal residue of ICSH-α was serine and that of ICSH-β was leucine. More recently, reinvestigation of the N terminus by the dansyl procedure[10] shows that ICSH-α has an NH_2 terminal phenylalanine and ICSH-β begins with serine. We have previously reported on the sequences of two peptides obtained by CNBr cleavage of ICSH-β which accounted for 39 of the 120 residues present[11] in ICSH-β. We wish now to report the results of sequence studies which allow us to postulate the entire linear amino acid sequence of both ICSH-α and ICSH-β.

Ovine ICSH, and its subunits ICSH-α and ICSH-β, were prepared by the procedures previously described.[5,12] Performic acid oxidations as well as reduction and alkylation of the hormone were performed by methods[13,14] reported earlier. CNBr reactions[15] were carried out in 70% formic acid. Completeness of performic acid oxidation, reduction, and alkylation and CNBr cleavage was determined by amino acid analysis of the products. Amino acid analyses[16] were performed with a Beckman amino acid analyzer Model 120 B.

Digestions with trypsin, chymotrypsin, and subtilisin were carried out in 0.1 *M* ammonium acetate of pH 8.3 for 8 hr at 37° (enzyme–substrate, 1:100 or 1:50). CNBr reaction mixtures were fractionated on columns of Sephadex G-50 in 20% formic acid (v/v). Enzyme digests were fractionated first on columns of Sephadex G-50, -25, and -15 in 0.01 *M* NH_4OH; further purifications were effected by paper chromatography in the system 1-butanol–acetic acid–water (4:1:5, v/v), or 1-butanol–pyridine–acetic acid–water (5:2:1:4, v/v) and by high- and low-voltage electrophoresis on paper in buffers of either pH 2.0 or 6.4. Purity of peptides was assessed by terminal group analysis,[10] quantitative amino acid analysis,[16] and by the fingerprint technique.[17] Sequences were established by the Edman[18]–dansyl[10] technique, the Edman subtractive method,[19] and kinetic studies of digestion with leucineaminopeptidase and carboxypeptidase.

The proposed structure of ICSH-α is shown in Chart I and that of ICSH-β in Chart II. ICSH-α consists

Chart I. Amino Acid Sequence of Ovine ICSH-α(CI)[a]



[a] CHO, carbohydrate moiety; ⇓, CNBr; ↑, trypsin; ↓, chymotrypsin; ↑, subtilisin.

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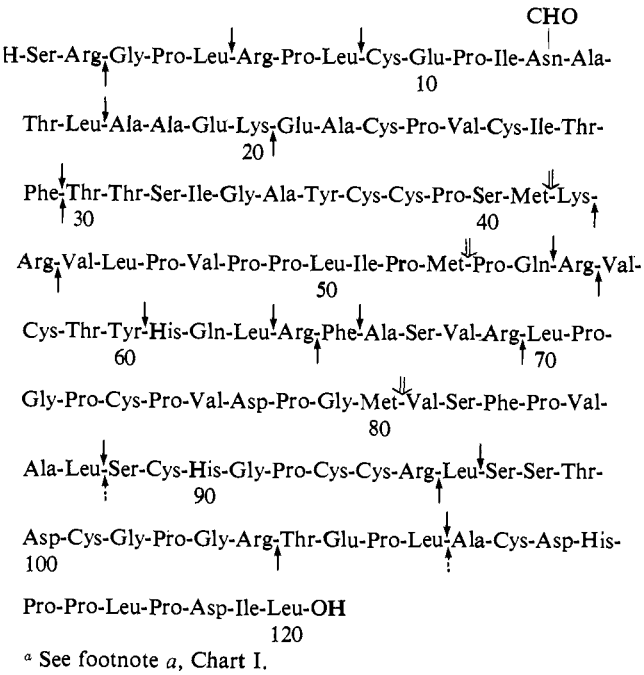
Chart II. Amino Acid Sequence of Ovine ICSH-β (CII)[a]



[a] See footnote *a*, Chart I.

of 96 amino acid residues. There are two carbohydrate moieties which are linked to asparagine residues in positions 56 and 82. Liao and Pierce[20] have recently shown that thyrotropin (TSH) consists of chemically dissimilar subunits as does ICSH, and one of the subunits, TSH-α, is very similar to ICSH-α. Pierce, *et al.*,[21] have also determined the amino acid sequences of the TSH subunits. Comparison of the structure of ICSH-α (Chart I) with that of TSH-α reveals that the two are nearly identical. This is all the more remarkable when one considers that the TSH was of bovine origin while the ICSH in this study is of the ovine species.

ICSH-β (Chart II) consists of 120 residues in contrast to the 96 of ICSH-α, contains but one carbohydrate moiety (residue 13), and possesses 12 half-cystine residues compared to the 10 in ICSH-α. The sequence of the peptides previously described[11] is located in the region of 42–80. Liu, *et al.*,[22] in a preliminary communication have also postulated a structure for the ICSH-β subunit. There are certain differences worthy of note. Their structure lacks the carboxyl terminal sequence · · · Ile-Leu present in our formulation. Also, their structure lacks two half-cystine residues (no. 38 and 93) as well as a threonine residue (no. 28) found in the structure presented in Chart II. Additionally, Liu, *et al.*,[22] postulated the amino terminal serine residue to be acylated.

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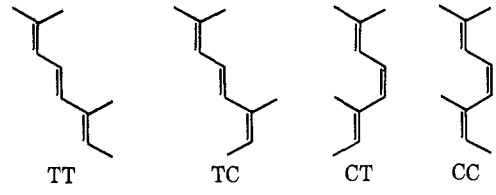
Photochemistry of Polyenes. I. A Comparative Study of Cis–Trans Isomerization from the S_1 and T_1 States of a Conjugated Triene (Alloocimene)

Sir:

Studies of the photochemical reaction of conjugated trienes have been largely limited to direct irradiation leading to electrocyclic or sigmatropic rearrangements.[1] The cis–trans isomerization reaction has been studied only in a cursory manner.[1d-f] In this communication, we wish to report some of our findings from the isomerization studies.

The triplet state energies of the trans and cis isomers of 1,3,5-hexatriene are 47 and 48 kcal/mol, respectively.[1e,2] Therefore, triene triplet states can be readily populated with most of the conventional photosensitizers. On the other hand, isomerization by direct irradiation can be effected by employing light of 2537 Å.[1]

The presence of several possible isomers in a substituted triene complicates cis–trans isomerization studies. The most complex case would be a triene with different substituents at the terminal positions giving a maximum of eight isomers. To reduce complexity while not sacrificing interesting information we studied the alloocimene, 2,6-dimethyl-2,4,6-octatriene, system where only four geometric isomers are possible. All four isomers are readily isolable and their structures known.[3]



Isomers TT and TC are present in commercial alloocimene, thus most readily available. Isomers CT and CC can be obtained from an irradiated[4] mixture of the triene. By preparative glc all four isomers have been isolated in at least 90% purity. Direct and sensitized (by benzophenone) irradiation of dilute solutions of pure isomers were performed. Progress of the reactions was followed by glc analyses of aliquots

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(4) The photostationary state composition varies with triene concentration. At 0.05 *M*, the stationary mixture (sensitizer–benzophenone) contains 18 and 15% of CC and CT, respectively.