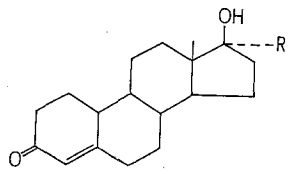
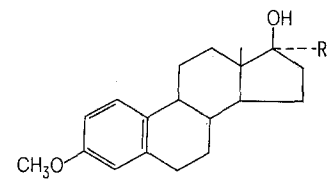
Table I. Progesterone-like property of norethindrone allenologs



R	No. of rabbits	MED[a] (mg)	Relative potency
$-C\equiv CH$ (norethindrone) III	55 (6)[b]	0.05	100
$-CH=C=CH_2$ IV	42 (7)	0.0025	2000
$-C(CH_3)=C=CH_2$ V	51 (5)	0.005	1000

[a] MED, daily dose of the compound which if administered s.c. for 5 days produced a minimal effective response in the Clauberg test. [b] Dose levels used to establish MED.

Table II. Estrogenic effects of mestranol allenologs



R	No. of rats	ED_{50} (mg)	Relative potency
$-C\equiv CH$ (mestranol) VI	137 (4)	0.003	100
$-CH=C=CH_2$ VII	24 (3)	0.010	33
$-C(CH_3)=C=CH_2$ VIII	82 (4)	0.015	20

additive over several of those increases known to be obtainable with certain 'skeletal' modifications. The necessity of the direct attachment of the unsaturated side chains in IV and V – i.e., of the α-allenyl carbinol nature of these compounds – is dramatized by the almost complete lack of activity of the related β-allenyl carbinol, R = $-CH(CH_3)-CH=C=CH_2$, this having a saturated carbon atom inserted between the allene moiety and the nucleus.

In contrast to the above picture, the estrogenic potency (as determined from the rat vaginal smear assay[11]) of the mestranol type is only slightly affected (in fact, is reduced) by the acetylene → allene modification (Table II).

Presently we have no biochemical explanation for this allene enhancement. It seems improbable that such a relatively small steric difference as that between acetylene and allene could account for so considerable an increase in activity. It is perhaps more attractive to speculate that the greater (and different) chemical reactivity of the allenyl carbinols in the laboratory[12] is carried over to the physiological system and that a chemical reaction with a receptor protein becomes of prime importance[13].

It is, at any rate, tempting to conclude: would the allene side chains have been discovered first, the now ubiquitous acetylene would have hardly acquired such an exclusive position in the field of contraceptive progestins[14].

Zusammenfassung. Die Einführung einer Allengruppe an Stelle der üblichen Acetylen-Seitenkette in konzeptionsverhütenden 19-nor-Testosteronderivaten ergibt eine überraschende Steigerung der gestagenen Wirksamkeit. Eine analoge Modifikation typischer Östrogene wie Mestranol beeinflusst die östrogene Wirksamkeit nur unwesentlich.

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[12] E. GALANTAY, I. BACSO, D. HABECK and R. V. COOMBS, Symposium on Acetylenes and Allenes (The Chemical Society, Nottingham, England, July 1971).
[13] A similar hypothesis, based on the differences in chemical reactivity of an acetylene-allene pair, was recently forwarded by K. BLOCH et al. (K. ENDO, G. M. HELMKAMP JR. and K. BLOCH, J. biol. Chem *245*, 4293 (1970)) to explain the qualitatively different behavior of these compounds toward the enzyme system β-hydroxydecanoyl thioester dehydrogenase.
[14] The essential message of the present paper – i.e., the recognition of a certain uniqueness inherent in the 17 α-allenyl side chain – was first communicated at the Symposium on Acetylenes, Allenes and Cumulenes, July 1970[12]. Very recently, the preparation and properties of some of these allenic steroids have been reported by other workers: M. BIOLLAZ, R. H. LANDERS, L. CUÉLLAR, P. CRABBÉ, W. ROOKS, J. A. EDWARDS and J. H. FRIED, J. med. Chem. *14*, 1190 (1970).

Partial Sequence of the Tyrosine Region of Neocarzinostatin

The antitumor protein antibiotic neocarzinostatin (NCS)[1,2] has unique biological properties[1,3,4] and is being clinically tested[5]. We have investigated its chemical properties[2,6,7] and are presently engaged in structural studies[8,9] of this protein which consists of 109 amino acid residues. NCS contains a single tyrosine residue which has been implicated as important for its biological activity. A partial sequence is reported herein of the tyrosine-containing peptide (T_2, possessing 46 amino acid residues) obtained by automated EDMAN degradation[10,11] after tryptic digestion of tetra-S-carboxymethylated NCS.

The 2 disulfide bonds were reduced by dithiothreitol in liquid ammonia solution followed by S-carboxymethylation through addition of chloroacetic acid[12]. A pH-stat-controlled (pH 8.2) incubation with dicyclohexylcarbodiimide treated trypsin (Serva, Heidelberg) (E/S ratio, 1:100), for 40 min at 25°C under nitrogen in the presence of 0.001 *M* Ca^{++}, gave 5 fragments which were separated by preparative paper chromatography (n-BuOH/CH_3COOH/H_2O, 4:1:5, upper phase). The PAULY reagent was applied to a guide strip to identify the tyrosine-containing peptide (T_2) which was then eluted with 0.1 *M*

NH_4OH and lyophilized. Peptide T_2 was further purified by preparative paper electrophoresis[13] at pH 6.5. It was finally chromatographed on a Sephadex G-50 column (2.5×190 cm) with $0.1\,M$ NH_4OH.

The peptide (T_2), thus obtained, reacted positively with the PAULY, SAKAGUCHI, EHRLICH, ninhydrin, and chlorine-KI-starch reagents. Amino terminal analysis by the Dansyl-method[14] gave a single valine residue. The homogeneity of peptide T_2 was further corroborated by two dimensional electrophoresis-chromatography which showed a single spot. Amino acid analysis[15] gave (46 residues): $Arg_{1.0}$CM-$Cys_{1.5}Asx_{5.5}Thr_{4.3}Ser_{4.1}Glx_{2.0}Pro_{1.1}Gly_{6.1}Ala_{9.0}Val_{6.2}Leu_{3.1}Tyr_{1.0}Phe_{1.0}$ and $Trp_{1.0}$. Tryptophan was determined spectrophotometrically[16]. Manual subtractive EDMAN degradation[17], carried out essentially according to the prodecure described by BLOMBÄCK et al.[18], gave the N-terminal tripeptide sequence Val-Ala-Gly-. Sequence analysis in the automated protein sequenator (BECKMAN, model 890), following previously described methods[10,11], gave the following result in twice repeated experiments: Val–Ala–Gly–Ala–Gly–Leu–Gln–Ala–Gly–Thr–Ala–Tyr–Asp–Val–Gly–Gln–Cys(CM)–Ala–( )–Val–Asn–Thr–Gly–Val–Leu–. No residue could be definitely identified at step 19 in either degradation, possibly due to the presence at this position of an amino acid (such as serine) giving rise to a labile thiohydantoin derivative. No other difficulties were observed in the sequenator study. Work on the complete sequence of neocarzinostatin is in progress and will be published elsewhere[19].

Zusammenfassung. Das antitumoraktive Protein-Antibiotikum Neocarzinostatin wurde mit Dithiothreit in flüssigem Ammoniak reduziert und mit Chloressigsäure alkyliert. Tryptische Spaltung des tetra-S-carboxymethylierten Proteins ergab 5 Fragmente. Die Sequenz von 25 Aminosäureresten im tyrosinhaltigen Fragment H_3 wurde durch EDMAN-Abbau im automatisierten Sequenator ermittelt.

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The Influence of Vitamin E on the Expenditure of Vitamin A from the Liver

Since the first communication on the subject by MOORE[1] it is generally recognized that vitamin E is necessary for optimal utilization of vitamin A.

Studies with chicks and rats receiving vitamin A in the form of cod liver oil (DAM et al.[2,3]) showed that more vitamin A was stored in the liver when the diet contained D, L-α-tocopheryl acetate than when the diet was vitamin E deficient, and, further, that methylene blue and other redox dyes had an effect on the storage of vitamin A in the liver similar to the effect of vitamin E. It was, therefore, held likely that the sparing action on vitamin A exerted by vitamin E is simply due to inhibition of the autoxidation of polyunsaturated fatty acids which, if unchecked, would lead to destruction of vitamin A.

The present studies, the details of which will be reported elsewhere, show that vitamin E has a sparing effect on the vitamin A content also when the diet does not contain fatty acids.

53-day-old chicks received for 13 days a 'starter ration' containing no vitamins A and E. During the last 6 days of this period, each chick received by mouth equal doses of retinyl acetate. Analysis (method as in reference[4]) of the livers of 10 chicks killed on the 14th day showed that the average amount of retinol per liver was 8.37 mg. The remaining 43 chicks were divided into 4 groups and given the vitamin A deficient diets indicated in Table I for a period of 4 weeks, whereafter they were killed and their livers assayed for vitamin A. The average amounts of vitamin A (retinol) found in the livers from each group of chicks after the 4-weeks depletion period are indicated in Table I as percent of the average amount (8.37 mg) present immediately before the depletion period.

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