pendent of Na+; on the other hand, the influx of GlcNAc appears to be the expression of a simple process of diffusion.

Finally, in certain experiments in which everted sacs were used, an attempt was made to find out whether the absorption of glucosamine and GlcNAc involved modification of their molecular structure. With this aim in view, the glucosamine $1^{14}C$ or the GlcNAc $1^{14}C$ were only added to the mucosal liquid. After 1 h of incubation, the glucosamine and the GlcNAc of the mucosal and serosal fluids were separted from one another on a Dowex column 50 W × 8 H+ (200–400 mesh)[9]. It was possible to demonstrate that both the glucosamine and the GlcNAc are absorbed without modification of their molecular structure. It was thus excluded that the absorption of GlcNAc involved deacetylation.

In conclusion, the influx of the glucosamine into the intestinal cells is carrier mediated, independent of Na+ and takes place without active accumulation, GlcNAc is absorbed by a simple process of diffusion without deacetylation of the molecule.

Riassunto. È stato studiato l'assorbimento della glucosamina e della N-acetilglucosamina utilizzando segmenti intestinali tagliati trasversalmente (strips) e sacchetti di intestino tenue di ratto. Il flusso di trasporto della glucosamina nelle cellule intestinali è mediato da carrier, indipendente dallo Na+ e procede senza accumulo attivo. La N-acetilglucosamina sembra, invece, essere assorbita per mezzo di un semplice processo di diffusione.

L'assorbimento, sia della glucosamina che della N-acetilglucosamina non comporta modificazione della loro struttura molecolare.

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25 November 1971.*

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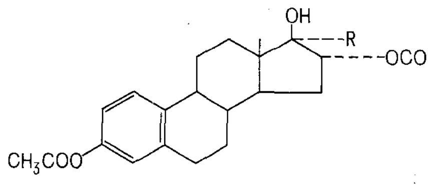
[9] G. TESORIERE, R. VENTO, D. MAGISTRO and F. DONES, Eur. J. Biochem. *19*, 289 (1971).

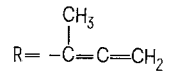
[10] E. L. JERVIS and D. H. SMYTH, J. Physiol., Lond. *149*, 433 (1959).

Allene versus Acetylene in Progestational Steroids

In the development of orally active progestational and estrogenic agents, the introduction of the acetylene side chain (17α-ethynyl) was of unique importance. Ethisterone (17α-ethynyl-testosterone) prepared in 1938, was found to be an orally active progestin rather than the expected androgen, and thirty-three years later we find that not only are virtually all contraceptive progestins on the market still of the ethynyl-nortestosterone type[1] but also that the most widely used estrogens (mestranol, ethynylestradiol) incorporate this same feature as well[2]. During these years innumerable attempts to find a side chain biologically superior to the original acetylene have resulted in no significant improvement[3]; all the advances being achieved through technologically demanding, expensive 'skeletal' modifications[4].

Some years ago, we made the surprising observation that the allenic compound I obtained as a side product in the preparation of II[5], showed activity in the rabbit deciduoma test[6] (a test in which a progestinestrogen combination or a steroid with a progestational-estrogenic profile would be active) whereas II itself behaved more as an 'impeded-estrogen', typical in fact of these estriol-type compounds.





I $R = -\overset{CH_3}{\overset{|}{C}}=C=CH_2$

II $R = -CH_2-C\equiv C-CH_3$

Suspecting that the allene side chain of I might have been responsible for the appearance of a progestational component in the activity and hoping that by attachment of the same allene to a 'progestational' skeleton highly active progestins could be obtained, we prepared[7,8] the 'allenologs' of several known ethynyl-nor-testosterone derivatives and submitted them to the Clauberg assay. Typical responses to the allenologs of norethindrone are shown in Table I.

The remarkable increase in activity found on progressing from acetylene to allenes was also observed in other related tests (e.g., antiestrogenicity[9], inhibition of ovulation[10]). Moreover these increases appear to be quasi-

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[1] Several representatives of the other important class (17α-acetoxyprogesterones) have been withdrawn from the market.

[2] For a recent review on acetylenic steroids, see D. ONKEN and D. HEUBLEIN, Pharmazie *25*, 3 (1970).

[3] For an example, see J. H. FRIED, T. S. BRY, A. E. OBERSTER, R. E. BEYLER, T. B. WINDHOLZ, J. HANNAH, L. H. SARETT and S. L. STEELMAN, J. Am. chem. Soc. *83*, 4663 (1961).

[4] For a recent review of progestational steroids, see V. PETROW, Chem. Rev. *70*, 713 (1970).

[5] E. GALANTAY, USP No. 3, 501, 510 of July 28, 1967.

[6] R. L. ELTON, Acta Endocr., Copenh. *51*, 543 (1966).

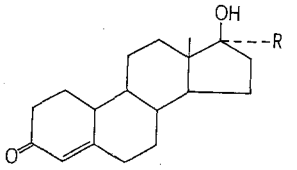
[7] A highly practical pathway for the preparation of the allenes of the type IV has, as the key step, the $LiAlH_4$ reduction of the quaternary Mannich salts, $R = -C\equiv C-CH_2-\overset{+}{N}R_3$: E. GALANTAY and D. HABECK, Belgian Patent No. 742,137. The preparation of the type V is exemplified in USP No. 3,541,210.

[8] An alternative pathway to these compounds, described by other workers in USP No. 3,392,165 and 3,392,166, appears to be in divergence to experimental results and its merits or otherwise, can be held in abeyance pending publication of full procedures and results.

[9] R. A. EDGREN, D. W. CALHOUN, R. L. ELTON and F. B. COLTON, Endocrinology *65*, 265 (1959).

[10] D. L. PETERSON, R. A. EDGREN and R. C. JONES, L. Endocr. *29*, 255 (1964).

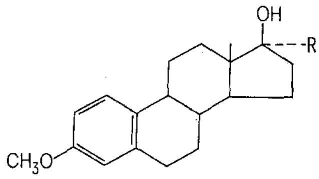
Table I. Progesterone-like property of norethindrone allenologs



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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Hanover (N.J. 07936, USA), 10 January 1972.*

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[11] R. A. EDGREN and D. W. CALHOUN, Am. J. Physiol. *189*, 355 (1957).

[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 $0.1 M$