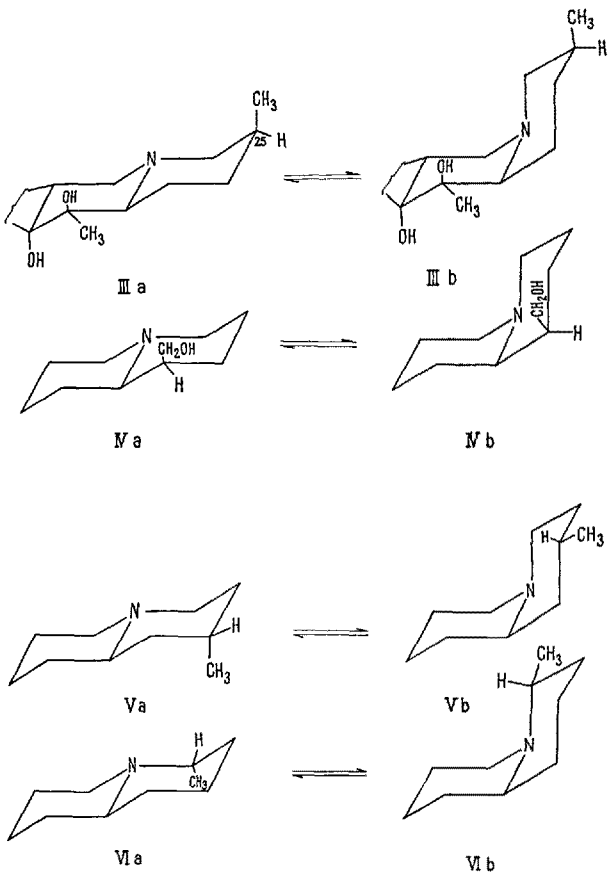
concluded that *trans*-2-methylquinolizidine (the prefix referring to the relative configuration of 10-hydrogen atom with respect to that at position 2) exists in the *trans*-fused conformation Va, whereas for *trans*-4-methylquinolizidine the *cis*-fused conformation VIb is preferred.



On usual conformational analysis basis, the equilibria Va–Vb and VIa–VIb should have similar reaction energies and the proportion of the *trans*-fused conformation should be similar in each of the equilibria[13]. The seemingly glaring difference in the two equilibria is difficult to explain even if it is allowed, for the sake of argument, that their reaction energies differ by about 1.6 kcal/*M* [$\Delta\Delta F = \Delta F$ (Vb–Va) $- \Delta F$ (VIb–VIa) = 1.6]. In such a case, simple calculations for 27°C show that at least 50% of Vb should be present at equilibrium, the proportion of VIa at equilibrium being assumed to be less than 5% (no absorption in 2800–2700 cm^{-1} region[5]). Apparently the I.R. and the N.M.R. criteria as such are able to detect only the presence of *trans*-fused conformations and are inadequate to exclude even substantial quantities of the *cis*-conformations. Alternatively, the simple conformational analysis developed on carbocyclic systems is not applicable to nitrogen systems and special steric or electronic effects are operative[14].

Zusammenfassung. Eine Abschätzung der relativen Stabilität der *cis*- und *trans*-Konformationen von Chinolizidin, Octahydropyrocolin sowie einigen ihrer Abkömmlinge wird durchgeführt. Unter Berücksichtigung des Einflusses des isolierten Elektronenpaars am Brückenkopf-Stickstoff ergibt sich eine grössere Stabilität für die *cis*-Verbindungen.

S. V. Kessar

Department of Chemistry, Panjab University, Chandigarh (India), October 9, 1961.

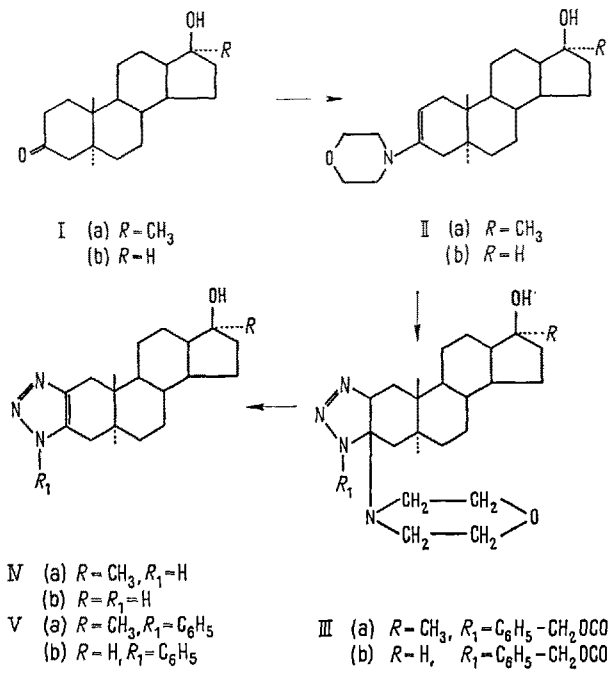
[13] Both VIa and VIb have an extra skew interaction as compared to Va and Vb.

[14] It is difficult to explain the observation of Moynehan[5] et al. that, as compared to *trans*-4-methylquinolizidine, the *trans*-fused conformation is favoured for *cis*-1-methylquinolizidine and *cis*-3-methylquinolizidine (both having an axial methyl group *cis* to the nitrogen lone pair), except on the basis that the presence of three hydrogens *trans* to the nitrogen lone pair makes the latter two detectable in lower concentrations.

A New Series of Potential Anabolic Steroids: [2,3-*d*]-Triazolosteroids

Fusco[1], in the course of an extensive research on enamines of alicyclic ketones, was able to demonstrate that different azides react with these compounds to give 1,2,3-triazoles. These results prompted us to extend this process to the enamines of some 3-ketosteroids in order to obtain steroidal molecules, in which ring A is condensed with a triazole ring.

The anabolic activities of some androstane derivatives, in which ring A is fused with various heterocyclic rings (pyrazole, isoxazole)[2–5], enhanced our interest in this



[1] We are indebted to Prof. Dr. R. Fusco for the information on his results prior to publication. Our work in this field is a direct extension of this information. The contribution of Prof. R. Fusco to this research is gratefully acknowledged.

[2] R. O. Clinton, A. J. Manson, F. W. Stonner, A. L. Beyler, Gr. O. Potts, and A. Arnold, J. Amer. chem. Soc. *81*, 1513 (1959).

[3] Belgian patent 580, 902.

[4] A. Arnold, A. L. Beyler, and G. O. Potts, Proc. Soc. exp. Biol. Med., N.Y. *102*, 184 (1959).

[5] G. O. Potts, A. L. Beyler, D. F. Burnham, Proc. Soc. exp. Biol. Med., N.Y. *103*, 383 (1960).

research. Furthermore, ZDERIC et al.[6], in a recent preliminary report, claimed 17α-methyl-androstane-2'-methyl-[3,2-b]-thiazole-17β-ol and some N-substituted 2-aminomethylene derivatives of 17α-methyl-dihydrotestosterone to be highly anabolic active.

17α-Methyl-5α-androstane-17β-ol-3-one (Ia) (dihydromethyltestosterone) was converted[7] to the 3-morpholyl-enamine (IIa) m.p. 209–216°; $[\alpha]_D$ + 6.6° (c = 2, $CHCl_3$) found: C 76.91; H 10.36; N 3.58. IIa when reacted with benzyl azidoformate[8] gave 17α-methyl-3-(N-morpholyl-5α-androstane-17β-ol-[2,3-d]-N¹-carbobenzyloxy-triazole (IIIa) m.p. 141° (dec.); $[\alpha]_D$ + 166° (c = 1, $CHCl_3$) found: C 70.00; H 8.46; N 10.48. Hydrogenation of IIIa with palladium on charcoal or lithium aluminum hydride gave *17α-methyl-5α-androstane-17β-ol-[2,3-d]-triazole* (IVa) m.p. 270–271° (Kofler); $[\alpha]_D$ + 26° (c = 0.63, CH_3OH) $\lambda_{max}^{CH_3OH}$ 225 mμ; ε 7.000, found: C 73.18; H 9.61; N 12.87; *IVa hydrochloride* m.p. 225–229°; $[\alpha]_D$ + 40.2° (c = 1, CH_3OH) found: C 65.41; H 8.74; N 11.72; Cl 9.45.

By reaction of IIa with phenylazide *17α-methyl-5α-androst-2-ene-17β-ol-[2,3-d]-N¹-phenyltriazole* (Va) was obtained; m.p. 288–291°; $[\alpha]_D$ + 53° (c = 1, $CHCl_3$), $\lambda_{max}^{CH_3OH}$ 230 mμ; ε 9800, found C 76.95; H 8.57; N 10.18.

Similarly, from the 3-morpholyl-enamine of 5α-androstane-17β-ol-3-one (IIb) (dihydrotestosterone) m.p. 180–190°; $[\alpha]_D$ + 31° (c = 2, $CHCl_3$); found C 76.57; H 10.40; N 4.06, 3-(N-morpholyl)-5α-androstane-17β-ol-[2,3-d]-N¹-carbobenzyloxy-triazole (IIIb) was obtained; m.p. 153–154°; $[\alpha]_D$ + 177.5° (c = 1, $CHCl_3$); found: C 69.67; H 8.44; N 10.51; reduction of IIIb gave *5α-androstane-17β-ol-[2,3-d]-triazole* (*IVb*) melting at 237–242° $[\alpha]_D$ + 75° (c = 0.751 CH_3OH), $\lambda_{max}^{CH_3OH}$ 225 mμ; ε 7.000; found: C 72.50; H 9.21; N 13.18, *IVb hydrochloride*, m.p. 258–266°, $[\alpha]_D$ + 94° (c = 1.12, CH_3OH); found: C 65.00; H 8.70; N 12.11; Cl 10.35.

IIb was reacted with phenylazide to yield *5α-androst-2-ene-17β-ol-[2,3-d]-N¹-phenyltriazole* (*Vb*); m.p. 306–311° (Kofler); $[\alpha]_D$ + 74° (c = 1, $CHCl_3$); $\lambda_{max}^{CH_3OH}$ 230 mμ; ε 9800; found: C 76.49; H 8.21; N 10.41.

A complete chemical and biological study on these compounds is being carried out and the results thereof will be published later.

Zusammenfassung. Es werden einige neue am A-Ring mit einem Triazolring kondensierte Steroide beschrieben. Diese werden durch Umsetzung der Enamine der 3-Ketosteroide mit Arylaziden oder Azidoformiaten und nachfolgende Abspaltung des basischen Restes hergestellt.

G. NATHANSOHN, E. TESTA, and N. DI MOLA

Research Laboratories of Lepetit S.p.A., Milano (Italy), August 7, 1961.

[6] J. A. ZDERIC, O. HALPERN, H. CARPIO, A. RUIZ, D. C. LIMON, L. MAGANA, H. JIMÉNEZ, A. BOWERS, and H. J. RINGOLD, Chem. and Ind. *1960*, 1625.

[7] F. W. HEYL and M. E. HERR, J. Amer. chem. Soc. *75*, 1918 (1953).

[8] The benzyl azidocarbonate, hitherto not reported in the literature, was obtained by reacting benzyl chlorocarbonate with sodium azide. It is a sufficiently stable oil, which can be purified by distillation *in vacuo*; b.p. 58°C/0.3 mm.

Structure and Pharmacological Actions of Eledoisin, the Active Endecapeptide of the Posterior Salivary Glands of Eledone[1]

It has been known for several years that acetone extracts of the posterior salivary glands of *Eledone* (*moschata* and *Aldrovandi*) contain a principle which possesses a powerful hypotensive action and potently stimulates extravascular smooth muscle[2]. This principle, called *eledoisin*[3], has now been isolated in a pure form and identified as the endecapeptide

Pyr-Pro-Ser-Lys-Asp(OH)-
Ala-Phe-Ileu-Gly-Leu-Met-NH_2[4]

Synthesis has fully confirmed the above constitution and aminoacid sequence[5].

Isolation procedure and determination of structure. 5368 g of posterior salivary glands, corresponding to approximately 10 000 pairs of glands obtained from 1450 kg of *Eledone*, were removed, at S. Margherita Ligure, from living animals and extracted twice with 3 vol of methanol. The combined filtered extracts were kept in the refrigerator and served as a standard crude extract for the isolation of the active principle.

The first step in the purification of eledoisin consisted in the absorption of the crude polypeptide dissolved in 95% ethanol on an alkaline alumina column and subsequent elution with descending concentrations of ethanol. According to experimental conditions, eledoisin was eluted, together with a large amount of aminoacids, by 60–40% ethanol, whilst biogenic amines were eluted by 90–80% ethanol.

Alternatively, eledoisin could be purified from a crude aqueous solution upon addition of serum albumin at pH 5. Salting out with ammonium sulphate resulted in precipitation of the protein complex which was collected by centrifugation, dialysed against water, then dissociated by treatment with trichloroacetic acid. The resulting protein-free eledoisin appeared to be freed from most of the low molecular weight contaminants. Further purification of the partially purified eledoisin obtained by any one of the above steps was carried out by ion-exchange chromatography on a column of Amberlite CG-50 in the H^+ form, performing the elution with a M ammonium acetate buffer at pH 8.4, and finally by a 160 transfer counter current distribution between 0.5 N acetic acid and *n*-butanol.

The above procedures gave a 1000–1200 fold increase in the activity/weight ratio and a yield of 20 to 40% of highly purified peptide.

Pure preparations appeared to be homogeneous on paper chromatography and electrophoresis giving a single peptide spot associated with biological activity. On ascending chromatograms run with the *n*-butanol : acetic acid : water mixture (5:1:4), eledoisin had an Rf of 0.6. On electropherograms carried out with aqueous buffers it appeared to be poorly soluble and showed a very low mobility in the range of pH 2–12.

[1] Supported in part by a grant from the Rockefeller Foundation, New York.

[2] V. ERSPAMER, Exper. *5*, 79 (1949).

[3] V. ERSPAMER, Arzneimittelforsch. *2*, 253 (1952).

[4] Pyr- = Pyroglutamyl-.

[5] R. A. BOISSONNAS and ED. SANDRIN, Exper. *18*, 59 (1962).