bordent des canaux haversiens. Or le pourtour de ces cavités apparaît incomplet sur l'autoradiographie pour deux des ostéones radioactifs. L'image histologique de la coupe décalcifiée et colorée au bleu de méthylène à pH 4,8 (C) démontre un détail supplémentaire essentiel: la présence de Zn^{65} est liée à celle d'un liseré préosseux.

Ce liseré préosseux s'inscrit à l'intérieur des couches calcifiées, seules visibles sur la microradiographie. Ses affinités tinctoriales ont été étudiées dans le laboratoire de LACROIX[4]. C'est en périphérie de ce liseré, le long d'une «ligne-frontière» clairement indiquée par le bleu de méthylène, que la substance organique de l'os commence brusquement à se charger de sels minéraux. C'est cette limite périphérique des liserés préosseux qui a été décalquée et reportée à l'encre sur l'autoradiographie. Bien entendu, elle correspond au contour des canaux haversiens tel que le dessinent les rayons X. Si l'on néglige l'inévitable diffusion des traces autoradiographiques, on doit admettre que la radioactivité est localisée le long de la ligne-frontière.

Il est hors de doute que le Zn^{65} se dépose dans le tissu qui est sur le point ou en train de se calcifier. Ajoutons incidemment que le cartilage de croissance retient, lui aussi, du Zn^{65} (observations inédites). Certes cet élément n'est pas le seul à se comporter de la sorte. Qu'il suffise de citer le Ca^{45}, dont la distribution hétérogène est si bien illustrée par l'autoradiographie[5], et certains colorants vitaux, parmi lesquels les tétracyclines ont fourni des images fluorescentes très démonstratives[6].

Mais on a observé[7] que les autoradiographies au Ca^{45}, en particulier quelques jours après l'injection, présentent un voile diffus qui traduit sans doute des réactions d'échanges. De même, d'autres radioéléments utilisés jusqu'à présent dans l'étude du métabolisme osseux peuvent démontrer les dépôts de substance minérale, mais ils se retrouvent aussi dans la masse du tissu déjà formé[8]. D'autre part, si l'on cherche un moyen d'évaluer par des mesures externes l'intensité des phénomènes de calcification dans une région déterminée du squelette, on ne peut, bien entendu, recourir au plomb[9] ni aux tétracyclines.

Le zinc, dont les isotopes 65 et 69 émettent des rayons γ, offrirait le double avantage de marquer électivement les sites de minéralisation et de ne pas se fixer dans les tissus squelettiques déjà calcifiés.

Enfin, il n'est pas sans intérêt de mentionner que, chez un singe sacrifié trois mois après injection de Zn^{65}, la radioactivité appartient à des lamelles osseuses calcifiées et enfouies dans la profondeur du tissu. Le zinc s'incorpore donc dans l'os, mais il y reste soustrait aux échanges et masqué pour l'histochimie.

Summary. Five days after intravenous administration of Zn^{65} to young monkeys, autoradiograms of compact bone show that radioactivity is exclusively located at the frontier line, where preosseous tissue is becoming mineralized. Since no diffuse reaction can be detected at this stage in the pre-existing calcified bone, it is concluded that Zn^{65} is not exchanged with the constituents of bone tissue, but does indicate accretion of new mineral substance.

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[4] J. VINCENT, Arch. Biol. *65*, 531 (1954); *68*, 561 (1957). – P. LACROIX, in *Bone Structure and Metabolism* (Ed. Churchill, London 1956), p. 36. – C. P. LEBLOND, P. LACROIX, R. PONLOT et A. DHEM, Bull. Acad. Roy. Méd. Belg. *24*, 421 (1959).

[5] P. LACROIX, Bull. Acad. Roy. Méd. Belg. *18*, 489 (1953); Second Radioisotope Conference *1*, 134 (Ed. Butterworths, London 1954). – J. VINCENT, *Recherches sur la constitution de l'os adulte*, Thèse Université Louvain (Ed. Arscia, Bruxelles 1955).

[6] J. P. GHOSEZ, Arch. Biol. *70*, 169 (1959). – W. H. HARRIS, Nature *188*, 1038 (1960).

[7] J. S. ARNOLD, W. S. S. JEE et K. JOHNSON, Amer. J. Anat. *99*, 291 (1956). – J. H. MARSHALL, R. E. ROWLAND et J. JOWSEY, Radiation Res. *10*, 258 (1959). – R. PONLOT, *Le radiocalcium dans l'étude des os* (Ed. Arscia, Bruxelles, et Masson, Paris 1960).

[8] R. E. ROWLAND, Clin. Orthop. *17*, 146 (1960).

[9] T. SATO, Gunma J. Med. Sci. *2*, 183 (1953). – J. VINCENT, Rev. Belge Pathol. Méd. Expér. *26*, 161 (1957).

Stereochemical and Polar Effects in Bromination of Androstan-3-one Mannich Bases

In a previous report[1] we outlined the preparation of Mannich bases of 3-ketosteroids. In particular, the preparation and configuration of 17β-hydroxy-2α-pyrrolidinomethyl-5α-androstan-3-one (I) was established. This communication is concerned with the bromination of I and its 17β-propionate ester (II) under thermodynamically and kinetically controlled conditions.

Bromination of I (or II) under thermodynamic control (excess hydrogen bromide in glacial acetic acid) gave rise to only one product, 4α-bromo-17β-hydroxy-2α-pyrrolidinomethyl-5α-androstan-3-one hydrobromide (III), m.p. 200–202°, $\lambda_{max}^{C_2H_5OH}$ 282 mμ, strong carbonyl band in the infrared at 1728 cm^{-1}. Dehydrobromination of III with lithium chloride in dimethylformamide gave 17β-hydroxy-2α-pyrrolidinomethyl-4-androsten-3-one, which was identical with a sample prepared earlier in an unambiguous manner[1]. Contrary to the findings of DJERASSI[2] et al., on the bromination of 2α-methylcholestan-3-one under equilibrating conditions, we could not isolate, even in minute amounts, 2β-bromo-17β-hydroxy-2α-pyrrolidinomethyl-5α-androstan-3-one (IV). This may be due to the strong positive charge on nitrogen.

The bromination of I and its enol acetate was then carried out under kinetically controlled conditions (carbon tetrachloride and/or acetic acid containing sodium acetate). Once again, *only one* α-bromoketone derivative, m.p. 169° was isolated. Dehydrobromination of this substance gave a dihydropyranyl dimeric steroid similar to the one previously described[3] and a small amount of the Δ^1 derivative, m.p. 168–170° ($\lambda_{max}^{C_2H_5OH}$ 237 mμ, ε = 8000). Thus, bromination had occurred at C_2. The infrared absorption spectrum of this bromoketone showed one strong band at 1710 cm^{-1} and maximum absorption in the ultraviolet was found at 312–315 mμ. Therefore, the bromine is axially oriented leading to one of the conformations, each considered as the hydrobromide salt.

[1] G. DESTEVENS and A. HALAMANDARIS, J. org. Chem. *26*, 1614 (1961).

[2] C. DJERASSI, N. FINCH, R. C. COOKSON, and C. W. BIRD, J. Amer. chem. Soc. *82*, 5488 (1960).

[3] R. MAULI, H. J. RINGOLD, and C. DJERASSI, J. Amer. chem. Soc. *82*, 5494 (1960).

In view of DJERASSI's findings[2,3], *a priori*, the boat structure Vb, obtained by a conformation 'flip' of Va to relieve the unfavorable 1,3-diaxial interaction of the pyrrolidinomethylene-methyl groups and the dipolar interaction of bromine-carbonyl, should be the expected product. Also, according to the axial halo ketone rule such a compound is expected to give a negative Cotton effect. In fact, however, the optical rotatory dispersion curve of the bromoketone in question was characterized by a strong positive Cotton effect[4] (Figure).

This establishes unexpectedly but unequivocally that the kinetically controlled bromination product is IV. Models reveal that direct equatorial entry of bromine is quite difficult due to steric hindrance imposed by the large pyrrolidinomethyl group. As a result, in the presence of large steric factors at C_2 (larger than a methyl group and at least equivalent in 1,3-non bonded steric effect to the angular methyl group), the axial attack of the reagent to the half-hair of the enol is the *only* course the reaction can take in spite of the steric influence of the angular methyl group.

Since it is conceivable that the hydrogen bonding effect of the proton and carbonyl in IV aids significantly in the A ring maintaining the chair form, it was of interest (a) to remove this source of intramolecular bonding and in so doing to impose a greater steric factor at C_2 and (b) to study the bromination of such a compound. Consequently, 17β-hydroxy-2α-pyrrolidinomethyl-5α-androstan-3-one methobromide (VI), m.p. 232°, carbonyl absorption in the infrared at 1710 cm^{-1} was prepared in the usual manner. Bromination of VI under kinetic control with bromine in acetic acid containing sodium acetate gave a product (VII) m.p. 176–176.5°, whose infrared carbonyl absorption band was displaced to 1730 cm^{-1} and an inflection was observed at 284 mμ in the ultraviolet region of the spectrum. This suggests an equatorial bromine. That it was not the 4α-bromo derivative was unambiguously shown by preparing the methobromide salt of III, m.p. 205–206°. The two substances were found to be distinctly different. Thus, compound VII is the 2-bromo derivative whose spectral properties indicate boat structure.

Once again because of the steric factors, axial bromination leads to VIIa. However, in this case the absence of hydrogen bonding effects plus the strong diaxial 1,3-bromine-methyl interaction results in a conformation 'flip' to the more stable VIIb with equatorial bromine.

In summary, the above data serve to illustrate further the directional influence of steric factors in kinetically controlled brominations[5]. Thus, axial bromination under kinetic control occurs even when there is steric hindrance to axial approach of the reagent provided that the steric factors associated with α-substituents (in this case substituents at C_2 in 3-ketosteroids) are of such magnitude to prevent equatorial entry. However, the polar effect of the basic nitrogen may also be significant and further work along these lines is in progress to elucidate this matter.

Zusammenfassung. Mannich-Basen von 5α-Androstan-3-one wurden unter thermodynamisch und kinetisch kontrollierten Bedingungen bromiert. Die Konformation der unter diesen Bedingungen erhaltenen Produkte wurde stark durch die sterischen und polaren Eigenschaften des Substituenten in 2-Stellung beeinflusst.

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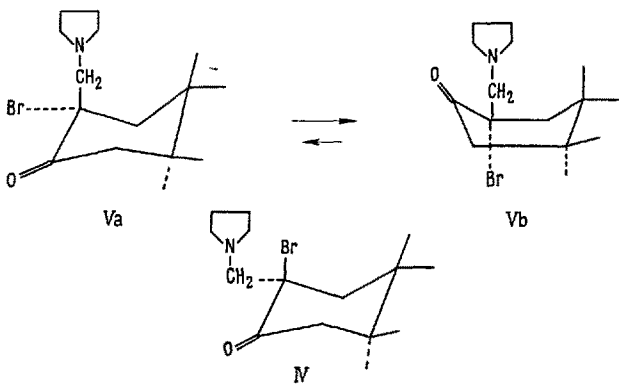
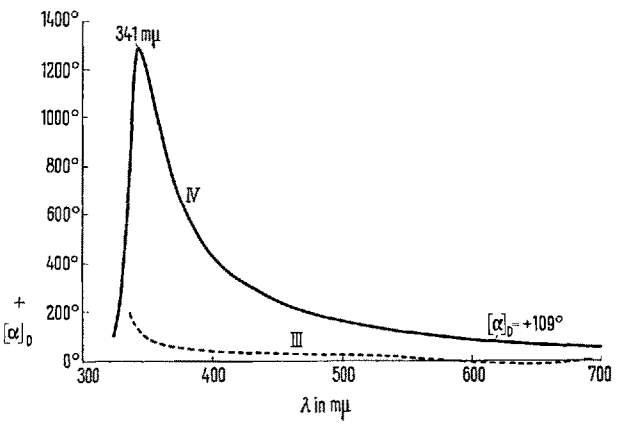
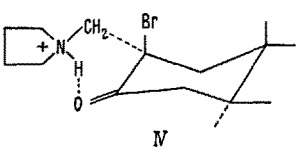
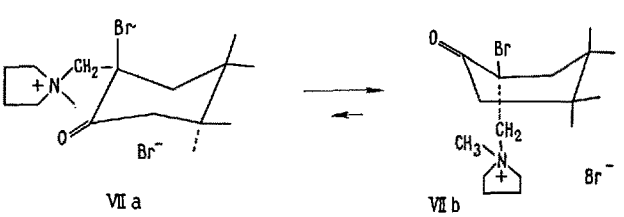
Chemical Research Division, CIBA Pharmaceutical Products Inc., Summit (New Jersey), April 24, 1961.









[4] The authors are indebted to Professor R. K. HILL, Princeton University, Princeton (N.J.), for carrying out this determination.

[5] R. VILLOTTI, H. J. RINGOLD, and C. DJERASSI, J. Amer. chem. Soc. *82*, 5693 (1960).