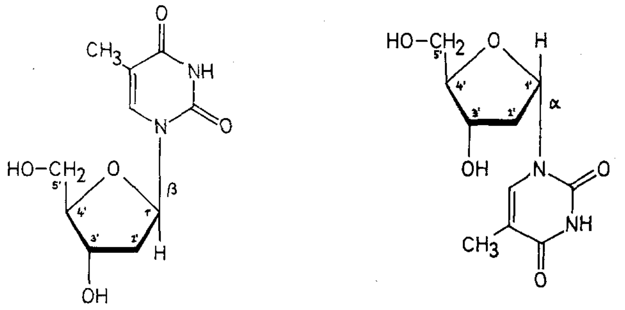
SPECIALIA

Nucleosides and Nucleotides. Part 5. The Stereochemistry of Oligonucleotides Consisting of 2'-Deoxy-α-D-Ribosides, a Study with DREIDING Stereomodels[1]

In the naturally occurring nucleosides and nucleotides, the ribose or deoxyribose and the bases are linked by a β-glycosidic bond (Figure 1). Hitherto only a few natural nucleoside derivatives have been known, in which the glycosidic linkage exhibits the anomeric α-configuration[2].



β–thymidine, βT_d[2]) α–thymidine, αT_d

Fig. 1. The 2 anomeric thymidines. Note that in β-thymidine the base at C(1') is oriented *cis* with respect to the hydroxymethyl group at C(4'), whereas in α-thymidine there is a *trans* relationship.

In 1955 KAPLAN et al.[3] described the isolation of α-NAD from commercial preparations of the coenzyme. α-NAD differs from the enzymatically active coenzyme by the α-glycosidic nature of the bond between ribose and nicotinamide. A few years ago, analogous compounds were isolated from *Azotobacter vinelandii* by SUZUKI et al.[4], i.e. α-NADP, α-NAD and two biogenetic precursors, α-nicotinic acid mononucleotide and α-nicotinic acid adenine dinucleotide.

A second group of α-nucleotides is found in the vitamin B_{12} family[5], vitamin B_{12} containing the 3'-phosphate of 1-(α-D-ribofuranosyl)-5,6-dimethyl-benzimidazole as structural element. Further substances related to vitamin B_{12} are characterized by modified benzimidazole units or by the presence of purines. Usually the latter are joined with the ribose moiety by N(7) [in nucleic acids always by N(9)]. In all these instances, the anomeric carbon atom of the ribose shows α-configuration. The conformation of some of these α-nucleosides was determined by SUNDARALINGAM[6] using X-ray diffraction.

Very recently DINGLINGER and RENZ[7] reported the isolation of 9-(α-D-ribofuranosyl)-adenine (α-adenosine) which was obtained after hydrolysis of the corrinoid factor C_x from *Propionibacterium shermanii*. This result represents the first case in which the anomer of a nucleoside occurring in the nucleic acids is found in nature.

It is noteworthy that only the β-anomeric nucleosides have been isolated from nucleic acids[8].

Due to the fact that they are not widespread in nature, α-nucleosides have not yet been studied very intensively. Yet, when nucleosides are chemically synthetized by

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[1] Part 4: R. GLASER, U. SÉQUIN and CH. TAMM, Helv. chim. Acta *56*, 654 (1973).

[2] Nomenclature and abbreviations used are in agreement with the CBN rules (Biochemistry *9*, 4022 (1970)), cf. also U. SÉQUIN and CH. TAMM, Helv. chim. Acta *55*, 1196 (1972).

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[8] Although the α-anomer of cytidylic acid has been detected in a yeast RNA hydrolyzate, it is not certain that this α-cytidylic acid is not an artifact produced during the alkaline hydrolysis; cf. T. UEDA and J. J. FOX, Adv. Carbohyd. Chem. *22*, 323 (1967).

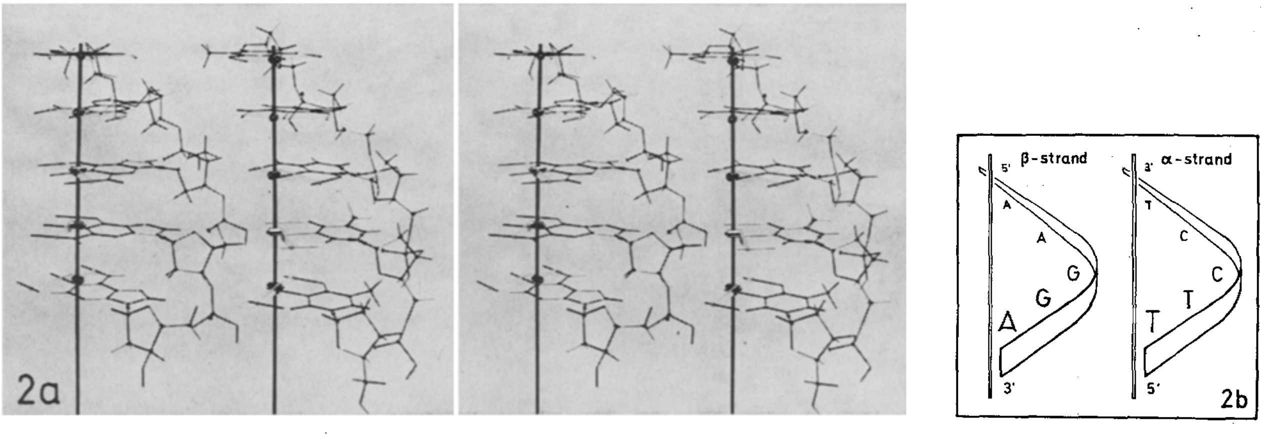

Fig. 2. The 2 pentanucleotides βd(A-A-G-G-A) and αd(T-T-C-C-T), a) Stereophotograph of the models[20]; b) Schematic.

condensing the base and sugar moieties, both, α- and β-anomers, are formed, the ratio depending on the experimental conditions[9]. In general the α-nucleosides obtained in this manner proved to be of little biological interest[10]. Very often they were not investigated further.

However, some interesting findings concerning α-nucleosides have been reported only very recently. 2′-Deoxy-α-thioguanosine exhibits carcinostatic activity[11]. Various tissue extracts are able to phosphorylate this nucleoside and it is incorporated into tumor DNA[12]. CHRISTENSEN et al.[13] investigated the biological activity in vitro of several 2-substituted 2′-deoxy-adenosines and found that also the α-anomers were able to inhibit the growth of certain organisms.

The behaviour of α-uridine, 2′-deoxy-α-uridine and 2′-deoxy-α-cytidine, and of some derivatives, towards nucleolytic enzymes was studied by HOLÝ[14]. And for the first time the synthesis of 2 dinucleoside monophosphates containing an α-nucleoside unit was achieved, i.e. U-αU and G-αU.

Polynucleotides with α-oriented bases. As mentioned above, nucleic acids contain exclusively β-nucleotides. Consequently no naturally occurring polynucleotides are known in which one or all nucleotide units possess the α-configuration. Therefore the chemical, physical and biological properties of such biopolymers are not known.

In this paper, studies are reported using DREIDING stereomodels which should demonstrate whether oligonucleotides consisting of α-nucleotide units are capable of exhibiting a secondary structure similar to that of the nucleic acids, featuring base pairing, base stacking and helix formation.

Assumptions and procedure. The DNA-B-structure[15] (10 base pairs per turn, pitch 34 Å, base pairs perpendicular to helix axis) was constructed with DREIDING stereomodels[16]. Oligomers of only 5 nucleoside units were made; they correspond to half a turn of the helix and therefore are of sufficient length for demonstrating the principal possibilities.

With respect to the exact geometry of the DNA-B-structure[15,17], the following simplifications were made: 1. Angles and dimensions of the bases are not exactly those found by X-ray analysis, due to the fixed angles and bond length of the commercially available model units. 2. The exact conformation of the deoxyribose ring is neglected. 3. The exact conformation of the sugar phosphate backbone is also neglected. 4. The atomic volumes are neglected, only the centres of the atoms are represented.

The aim of this preliminary study is to find out whether it is possible to arrange an oligonucleotide chain consisting only of α-nucleotides in a manner similar to the DNA-B-structure. The helix should not be strained. Furthermore all nucleotides should have the *anti*-conformation, since this is the preferred conformation of mononucleotides, nucleic acids[15,18] and of those α-nucleosides, the conformation of which has been investigated[6].

If such a helical arrangement were possible for an α-oligonucleotide, the next question to be answered would be whether a complex formation by base pairing is possible with a second, complementary α-strand or even with a complementary β-strand.

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[10] cf. e.g. M. HOFFER, Chem. Ber. *93*, 2777 (1960).

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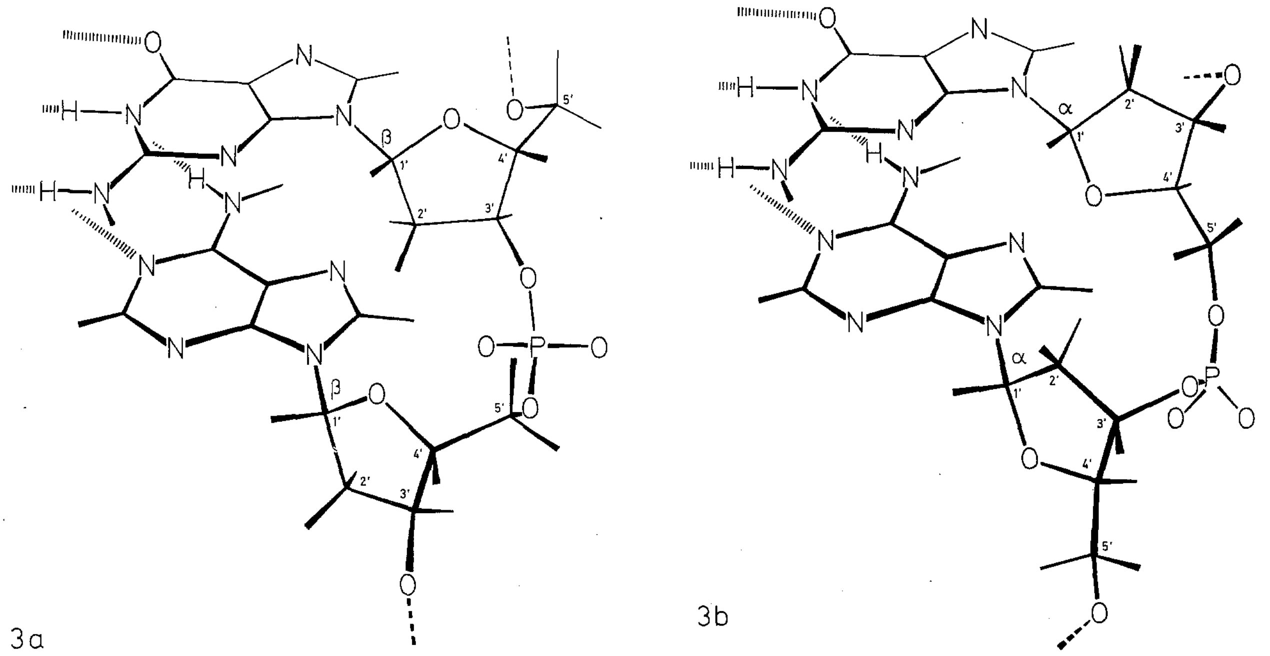

Fig. 3. a) Part of a β-oligonucleotide chain; b) part of an α-oligonucleotide chain, both in a similar arrangement as in Figure 2.

Comparison of the 2 oligonucleotides

Atoms of the β-strand	Corresponding atoms of the α-strand at the same location
Base	Base
C (1′)	C (1′)
C (2′)	O (4′)
C (3′)	C (4′)
O (3′)	C (5′)
C (4′)	C (3′)
O (4′)	C (2′)
C (5′)	O (3′)
O (5′)	P
P	O (5′)

Results. Figure 2 shows the two pentanucleotides βd (A-A-G-G-A) and αd (T-T-C-C-T) arranged in such a way that the 5′-end in the β-oligonucleotide stands up, in the α-oligonucleotide down. It can be seen that, indeed, an α-strand can be arranged in a manner very similar to a β-strand. The spatial positions of the atoms in the sugar phosphate backbone are practically the same in both chains, some groups of atoms having been interchanged. It is noteworthy that the 2 strands are of opposite polarity[19]. In the Table, the 2 oligonucleotides are compared; Figure 3 is a drawing of a part of the chains showing the details more distinctly.

Since a similar helical arrangement such as is observed with β-oligonucleotides, seems to be also possible for α-oligonucleotides, and since the position of the bases is identical in both strands, one might expect that an α-chain could form a complex with a complementary β-chain by base pairing. Note that in such a complex the 2 strands will exhibit the same polarity. The corresponding model was built as well: Figure 4 shows the complex of the 2 pentanucleotides αd (T-T-C-C-T) and βd (A-A-G-G-A).

In principle, even a double helix formed by 2 complementary α-oligonucleotides should be possible, but in this case the 2 strands will be of opposite polarity again. Figure 5 shows the model of a complex formed by αd (T-T-C-C-T) and αd (A-G-G-A-A).

The question whether the formation of double helices, as mentioned above, is possible with oligonucleotides containing both α- and β-nucleotides is likely to be answered by no. Since in a DNA-like arrangement, α-oligonucleotides show opposite polarity as compared with β-oligonucleotides, it would no longer be possible to maintain a uniform 3′-*p*-5′-linkage between the nucleoside

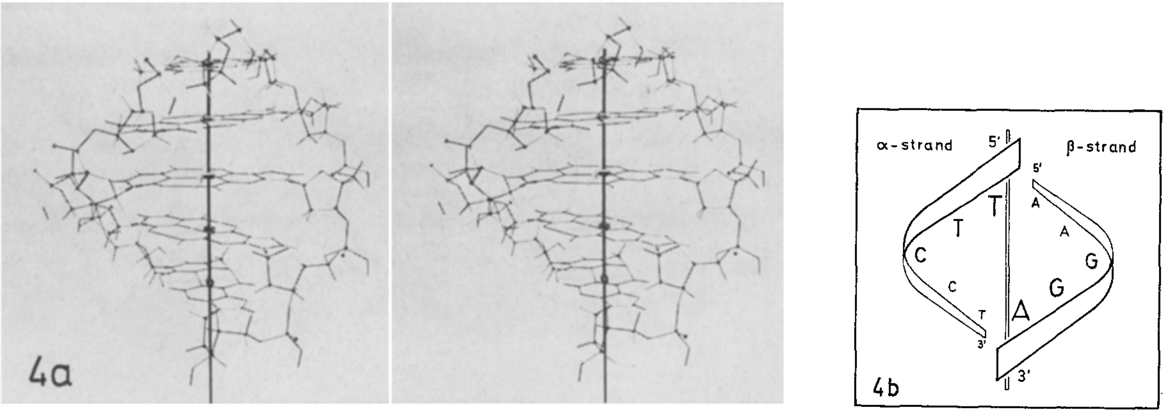

Fig. 4. Double helix formed by αd(T-T-C-C-T) and βd(A-A-G-G-A); a) Stereophotograph of the model; b) Schematic.

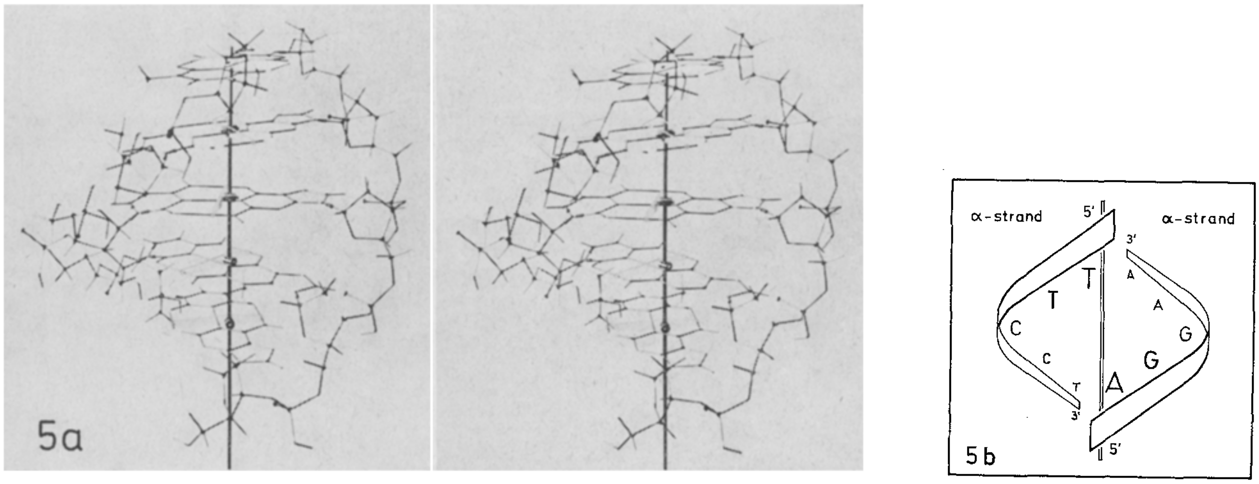

Fig. 5. Double helix formed by αd(T-T-C-C-T) and βd(A-G-G-A-A); a) Stereophotograph of the model; b) Schematic.

[19] Direction of the 3′→5′-phosphodiester linkage; cf. J. N. Davidson *The Biochemistry of the Nucleic Acids*, 6th edn. (Methuen & Co. Ltd., London 1969), p. 67, 204.

[20] The stereo pair may be viewed without optical aid or by means of a stereo viewer as supplied e.g. with the 'Stereo Supplement' to *The Structure and Action of Proteins* by R. E. Dickerson and J. Geis (Harper & Row, New York, Evanston & London 1969).

units and, at the same time base pairing and stacking. Thus, even a single α-nucleotide in a β-nucleotide chain is very likely to represent a serious 'mistake' in the chain.

Discussion. This investigation of molecular models demonstrates that the principles of base pairing and helix formation, which are essential for the propagation of the genetic information, are not exclusively possible with β-nucleotides. α-Nucleotides seem to be capable of exhibiting similar secondary structures. Thus one can assume that the replication of genetic material should proceed also with α-nucleotides. But such a replication appears to be possible only with nucleotides of uniform glycosidic configuration, since α- and β-nucleotides within the same strand interfer with helix formation.

Thus we are dealing with a similar phenomenon as is encountered for the amino acids and proteins: of two equally suitable stereoisomers, nature has chosen one, which thereafter is applied uniformly and consequently[21].

α-Oligonucleotides can prove to be useful tools for the investigation of enzymes related to the nucleic acid metabolism and protein biosynthesis with respect to their specificity to the α-glycosidic linkage. In an earlier paper of this series[22], it was shown that certain α-nucleoside 5′-phosphates are resistent to crude *Crotalus adamanteus* venom (acting as a 5′-nucleotidase). However, HOLÝ[14] observed, using a purified preparation of 5′-nucleotidase from *Crotalus adamanteus* venom, that the 5′-phosphates of α-uridine, 2′-deoxy-α-uridine and 2′-deoxy-α-cytidine were hydrolyzed.

The synthesis of oligonucleotides containing α-nucleoside units, their physical and chemical properties and their behaviour towards various enzymes are under investigation.

Zusammenfassung. Studien anhand von DREIDING Stereomodellen zeigen, dass die Bildung von helixartigen Sekundärstrukturen durch Basenpaarung und Basenstapelung nicht auf Polynucleotide mit β-ständig angeordneten Basen (Konfiguration wie sie in den Nukleinsäuren gefunden wird) beschränkt ist. Auch Polymere aus lauter α-Nucleotideinheiten scheinen dazu befähigt zu sein.

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18 June 1973.

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[21] Note that D- and L-amino acids are enantiomers, whereas α- and β-nucleosides are diastereomers.

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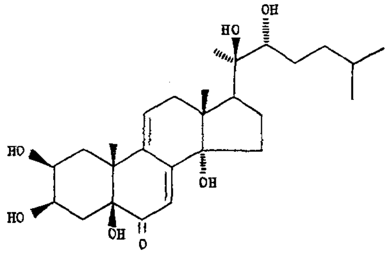
[23] Acknowledgments. The author thanks Prof. CH. TAMM for his continuous interest and for encouraging discussions. The investigation was supported by the 'Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung' (project Nr. 2.460.71).

The Structure of a New Phytoecdysone Kaladasterone: an Application of ^{13}C Magnetic Resonance Spectroscopy to Structural Problems

In the course of our investigation of family Convolvulaceae, we also studied constituents of several Indian Ipomoea species. From the seeds of one of them, which is locally known as Kaladana, we isolated together with previously known phytoecdysones (ecdysone, ecdysterone, makisterone A) also new compounds, muristerone A[1] (II), calonysterone[2], and kaladasterone. The determination of the structure of the last mentioned compound as I is reported herewith, and it is based on evidence from variety of spectroscopic as well as chemical data. We wish, however, to report some ^{13}C magnetic resonance studies which proved to be of importance in determining locations of functionalization of ecdysones.

Kaladasterone (I), $C_{27}H_{42}O_7$ (M^+ at m/e 478.2941, calc. 478.2930), m.p. 242–243° (methanol/acetone), $[\alpha]^{24}$ + 79.3° (metanol), shows in the IR- region absorptions at 1605, 1652, and 3200–3600 cm^{-1} (KBr), and an UV- absorption at λ_{max} (methanol 298 nm (ε 10800) characteristic for a doubly conjugated keto group. The PMR- and CMR- spectra closely resemble spectra of other phytoecdysones[3–5]. (The NMR-spectra were taken in deuterio-pyridine unless stated otherwise; δ_H as well as δ_C are given in ppm from TMS).

In fact, the PMR- spectrum in DMSO-d_6 exhibits signals of 5 methyl groups at δ 0.73; δ 0.86 (d, J $\simeq$ 7 Hz); δ 0.96, and δ 1.06, and of the usual olefinic proton at H_7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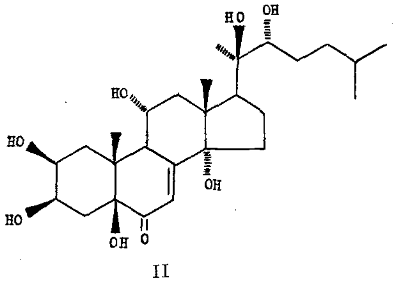
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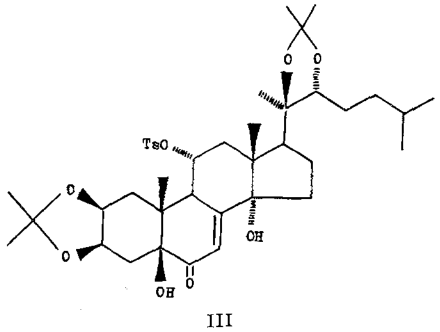
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