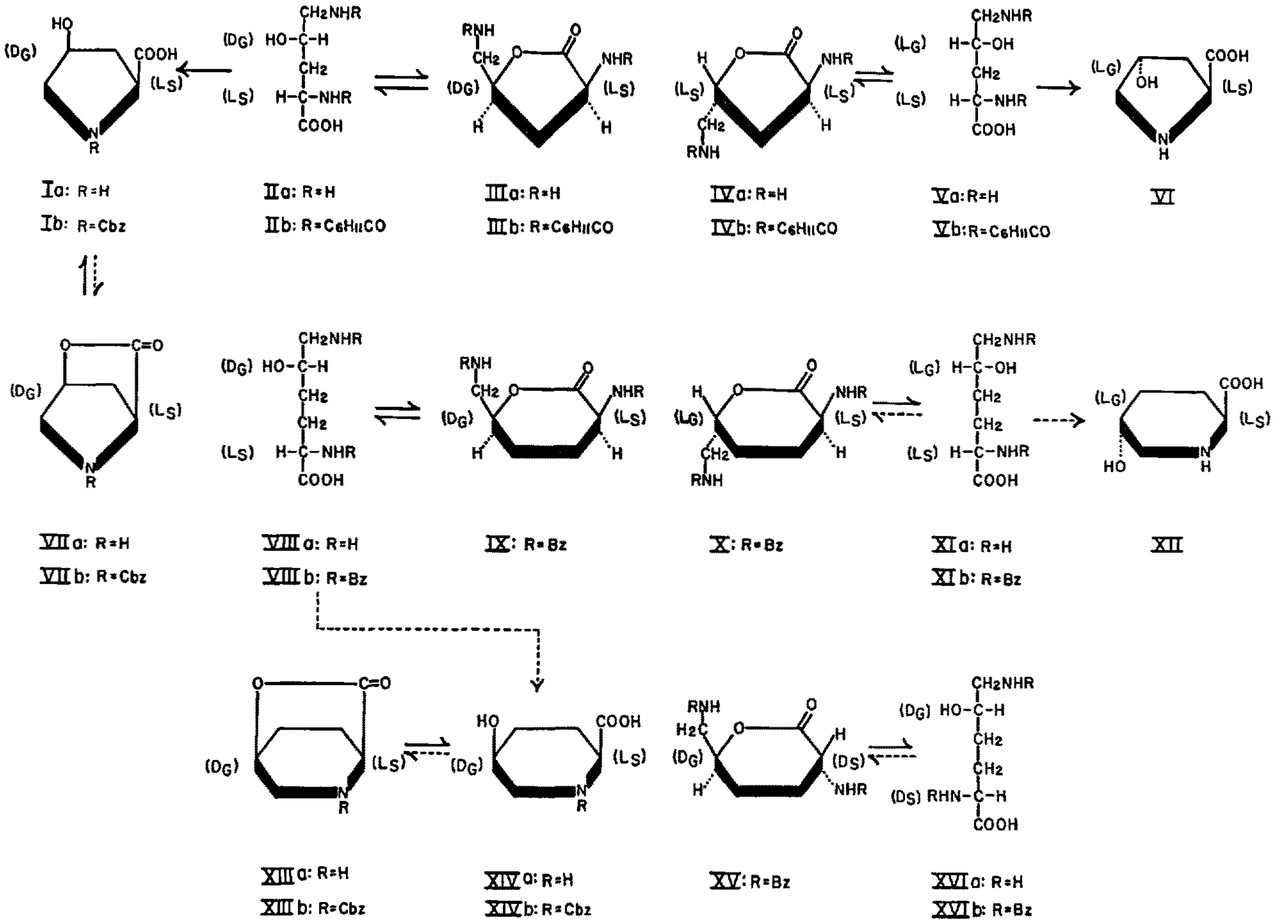

Configurations and correlations of γ- and δ-hydroxyamino acids and their lactones deduced from chemical and, for IX, X, XI, XV and XVI, rotational data. The designations D_G and L_G refer to D- and L-glyceraldehyde and D_S and L_S to D- and L-serine [cf. H. B. VICKERY, J. biol. Chem. *169*, 242 (1947); Chem. Eng. News *25*, 1365 (1947)].

With our increasing understanding of the structure of collagen[16] the confirmation of the absolute configuration of δ-hydroxy-L-lysine is of considerable importance.

It is a pleasure to thank Mrs. EVELYN G. PEAKE for the measurement of the rotations.

B. WITKOP

National Institutes of Health, Washington 14, D. C., July 27, 1956.

Zusammenfassung

Die Anwendung von Hudsons Lakton-Regel auf eine Reihe von Laktonen von γ- und δ-Oxyaminosäuren bestätigte die unabhängig auf chemischem Wege bewiesenen Konfigurationen an den γ- und δ-Kohlenstoffatomen. Im Falle des erstmalig dargestellten Laktons des α, ε-Dibenzoyl-δ-hydroxy-D-lysins führte die Anwendung der Regel zur *erythro*-Struktur XV, und folglich für natürliches δ-Oxylysin aus Collagen zur Formel XIa, deren Beweis auf chemischem Wege noch aussteht.

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lactone hydrochloride (i), whereas the hydrobromides of the lactones IIIa, IVa, VIIa and XIIIa show rapid mutarotation with the rate of ring-opening increasing qualitatively in the order IIIa ≅ IVa < VIIa < XIIIa.

[16] A. RICH and F. H. C. CRICK, Nature *176*, 915 (1955).

Electrochemical Behaviour of β-Carotene with Strong Acids

In previous communications we dealt with the electrolytic behaviour of some carotinoids in strongly acid media[1]. We now proceeded to examine whether it was possible to obtain "blue" solutions of β-carotene with other strong acids beside sulfuric acid, which was known up to now, by choosing suitable common solvents for acid and carotene. We succeeded in doing so and were thus able to examine the electrolytic behaviour of these blue solutions as well.

Our apparatus was essentially the one described[2], with the difference that the inverted V-tube was immersed into vessels of an internal diameter of 14 mm together with the Pt electrodes.

Hydrogen chloride in glacial acetic acid slowly turned blue with a drop of carotene in benzene, and the colour migrated towards the cathode. Presence of some water did not alter the result. Its total exclusion with acetic anhydride yielded a blue solution only if air was excluded also. As in all subsequent cases, the colour gradually changed and faded at last after some time: the mobilities and in some cases even the direction of migration were apt to change with this change of colour. We are evident-

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[1] F. KÖRÖSY, Exper. *11*, 342 (1955); Magy. kém. Foly. *61*, 155 (1955).

[2] F. KÖRÖSY and G. SZÉKELY, Magy. kém. Foly. *58*, 174 (1952).

ly dealing with a series of coloured derivates—isomers and/or homologues—which are formed as the 11 double bonds react beside and one after another.

Trichloroacetic acid in concentrated aqueous solution dissolves carotene with a blue colour; in isobutylic alcohol, the colour is green. Both migrate towards the cathode.

Formic acid in glac. acetic acid or butyl alcohol yields blue, cationic carotene solutions. There appears to be at least one second coloured compound in solution, because a faint grey-blue zone remains stationary where the bulk of the blue colour has moved away.

Perchloric acid reacts in a remarkable way: if shaken with a benzene solution of carotene, the latter, not the former turns blue. So far as I know, this is the first epiphasic, lipophilic blue compound of a carotinoid with a strong acid. A second case is described in my subsequent note in this same issue. If the carotene solution is not extremely dilute, a solid with golden lustre separates from the blue benzene solution[3]. This blue benzene solution does not migrate in an electric field.

However, 5 vol.% $HClO_4$ in acetic acid or 20 vol.% in butyl alcohol yielded cationic blue solutions which changed their direction of migration twice in the course of some hours. Higher concentrations (of the 67% $HClO_4$ used) immediately gave anionic products.

Phosphoric acid slowly evolves a blue-green colour with carotene in butyl alcohol; this moves to the anode.

Sulfuric acid[1] also reacted quite differently according to its concentration and to the time of reaction. Thus 2·5 vol% in acetic acid gave a blue, cationic solution, whereas $H_2SO_4 \cdot H_2O$ with about 20% methanol yielded a blue colour that moved to the anode. Some combinations did not show migration at all, at least in the beginning. After a time violet and brown colours developed and the tendency became anionic.

To sum up: the ions formed with monobasic strong acids and β-carotene are primarily, that is in the beginning and at lower concentrations of acid, all cations. Polybasic acids yield sometimes anions, perhaps because they are attached to the molecule and make it anionic by their second dissociation. However, as seen with small amounts of sulfuric acid in acetic acid, the formation of cations may be the primary event in this case also. It is not clear from this point of view how anions could have been generated in some cases by the monovalent perchloric acid. It may be that anions and cations can be bound with alternating strength to the conjugated system.

I gratefully acknowledge the help of the Hungarian Academy of Sciences, the accomodation provided by Prof. G. SCHAY and the assistance of Mr. G. SZÉKELY. A detailed account will appear in ths Acta Chimica Hungarica.

F. KÖRÖSY

Department of Physical Chemistry, Technical University, Budapest, Hungary, February 6, 1956.

Zusammenfassung

Die Einwirkung von starken Säuren auf β-Carotin in geeigneten Lösungsmitteln führte zur Bildung von blauen Farbstoffen. Bei Anwendung von mono- und polybasischen Säuren wanderten diese neuen Stoffe meistens kathodisch, wenn die Säurekonzentration schwach war. Manchmal, vor allem bei stärkerer Konzentration oder nach längerem Stehen der Lösungen, wanderten sie anodisch.

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[3] *Note added in the proof:* This solid is diamagnetic.

Action of Sulfuric Acid on β-Carotene

Concentrated solutions of β-carotene in benzene were shaken with ice-cold $H_2SO_4 \cdot H_2O$ under CO_2 and the blue solutions were decomposed with ice-water as quickly as possible. The yellow products were taken up in benzene, washed, dried and evaporated *in vacuo* to dryness. They were dissolved in benzene, and/or benzene petrolether mixtures, and chromatographed on a hydrated lime column or on a composite column made of a saccharose, a calcium carbonate and a hydrated lime section. We used petrolether (b.p. 60–70° C) or a mixture of the latter with ½ parts benzene as developers.

The sugar did not retain anything, the calcium carbonate layer retained an ochre-brown zone at its upper end, the rest developed several zones within the lime section. Development with petrolether split the mixture into more zones than was the case with benzene, especially at the bottom of the column. An upper red, thin zone was followed by a thin ochre one, then came a deep yellow-ochre, a thin red and two yellow zones one after another.

None of the zones showed the spectrum of β-carotene, despite the fact that the sulfuric acid reaction lasted only for 3 min. in one case. The boron trifluoride blue complex decomposed by ZECHMEISTER[1] did not behave in the same way; he could regenerate carotene if the reaction-time was only a few minutes. The lowest zone showed spectral maxima in CS_2 at 498, 467 and 440 mμ which correspond to a conjugated chain of 10 double bonds: 1 less than carotene. As no sulfur was detected in the eluted compound, a hydrolysis of the primary addition product must have occurred resulting in addition of one H_2O on a terminal double bond or in ω, ω'-position as in ZECHMEISTERS BF_3 experiments[2]. The other zones were examined in pentane solution and showed maxima at 426 (faint), 400, 375 and 345 mμ respectively. The zones did not seem to be sufficiently pure; their maxima were at the same wavelength irrespective of the fact that they belonged to distinct chromatographic zones of the column, but the relative heights varied from zone to zone. The maxima were shifted by 25–30 mμ towards longer wavelengths in CS_2. The eluted and concentrated carotinoids of each zone were brought once more into reaction with concentrated sulfuric acid. The top zone gave a violet colour, changing to pink. The red zone yielded a blue product, which was soluble in the benzene phase and left the underlying acid uncoloured. A similar case with β-carotene and perchloric acid was described in our preceding communication. These lipophilic blue acid-compounds of carotinoids seem to be absolutely unprecedented. The lower zones behaved normally in that they gave blue sulfuric acid solutions which changed to pink.

The fractions were neither sufficient nor pure enough to crystallize, but a generous gift of 20 g synthetic β-carotene from the Hoffmann La Roche Co., to whom I am very much obliged, will be of great help in this direction. Preliminary elementary analysis revealed that they contain oxygen.

I am about to isolate the "blue" compounds and separate them from each other if possible, to examine their decomposition products and general chemical, physical and magnetic properties.

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[1] L. ZECHMEISTER, Exper. *10*, 1 (1954). – L. WALLCAVE, J. LEEMANN, and L. ZECHMEISTER, Proc. nat. Acad. Sci. *39*, 604 (1953).

[2] L. WALLCAVE and L. ZECHMEISTER, J. Amer. chem. Soc. *75*, 4495 (1953).