Zusammenfassung

Die optischen Drehungen einiger 20α, 25D; 20α, 25L; 20β, 25D; und 20β, 25-L-Sapogenine wurden bestimmt. Die ersten drei Serien gaben übereinstimmend linksdrehende Werte, aber die letztere Gruppe erwies sich als rechtsdrehend.

Die Struktur der vier Serien der Sapogenine folgte aus der Analyse dieser Befunde. Der mögliche Mechanismus bei der Entstehung dieser Verbindungen aus Pseudosapogeninen wurde besprochen. Die Autoren gelangen zum Schluss, dass sterische Faktoren an C_{20} und C_{25} die Richtung der Ringschliessung beeinflussen.

Electrolytic Behaviour of Some Carotenoids in Strongly Acid Media

Some time ago, in the course of quite a different investigation[1], we dissolved carotene in isobutylalcohol which was saturated with a solution of 25% sulfuric acid in water, and we electrolysed this solution in an inverted V-tube[2] between two beakers containing 25% sulfuric acid in water into which anode and cathode were immersed. A bluish-green compound migrated towards the cathode and accumulated at the cathodic end of the isobutylalcohol layer. L. Cholnoky, to whom we are indebted for the gift of carotene and for discussions told us, on inspection of the experiment, that his carotene probably contained epoxides and he thought their salts were involved in the electrolytic migration. As a matter of fact, the yellow carotene itself did not migrate.

To identify the green, ionized compound, we prepared authentic β-carotene epoxides with perphthalic acid following Karrer's[3] method and resolved the resulting mixture by chromatography on a Ca/OH/$_2$ column with petrol-ether. Eight different zones were isolated and zones 6. and 7. were identified as Karrer's luteochrome and β-carotene-diepoxide respectively. The maxima of their absorption spectra in carbon disulfide were: 454 and 480 mμ for luteochrome (Karrer's values: 451 and 482 mμ) respectively 470 and 502 mμ for β-carotene-diepoxide (Karrer's values: 470 and 502 mμ). Both compounds were dissolved in the acidified isobutyl alcohol mentioned above. The initially yellow colour of the solutions turned gradually into blueish-green, as described by Karrer, in chloroform containing hydrochloride acid. These green compounds migrated towards the cathode in the above-mentioned inverted V-tube apparatus, applying 90 V to the 3 cm portion of the tube (internal diameter 2 mm) which was filled with the isobutyl alcoholic solution. The current was 5 mA. The green compound did not pass into the aqueous sulfuric acid but accumulated at the cathodic end of the alcoholic phase; it followed the current after reversal of the electrodes.

Other chromatographic fractions of the oxidized carotene behaved similarly, although their colour, which developed after a time in the acidified alcohol, varied between lavender-blue, violet and green. The coloured compound in some cases weakly passed the phase border into the aqueous acid at the cathodic side.

The cathionic behaviour of these carotenoids may be due either to their loosely bound π-electrons or to the formation of oxoniumsalts on the ring-oxygen atoms.

To test these hypotheses, we performed some further experiments. First we tried to electrolyse the chloroform solution of the blue product from carotene and $SbCl_5$ of the Carr-Price reaction, but failed to detect any migration of the blue boundarise between coloured and uncoloured chloroform. This colour reaction is given by carotenoids irrespective of whether they are pure hydrocarbons or contain oxygen. They are held to be dative compounds of the electron-donor carotenoids and the acceptor Lewis-acids ($SbCl_5$, $SbCl_3$, $AlCl_3$, BF_3, etc). In our case the dative compound evidently did not dissociate into positive and negative ions in chloroform solution.

Then we investigated the deep blue compounds which are formed from carotenoids and strong acids. It is well known that the hydrocarbon carotenoids yield these blue compounds with concentrated sulfuric acid, while carotenoids containing oxygen, e.g. violaxanthine, react even with dilute hydrochloric acid. Not much seems to be known, however, about these compounds.

We separated α- and β-carotene chromatographically from each other and examined their reaction with sulfuric acid of varying concentration. They dissolve in concentrated acid to a beautiful peacock-blue solution which however fades through ultramarin to an unsightly violet. Using sulfuric acid diluted with increasing amounts of water, we found that the peacock-blue colour is relatively stable at and beyond 15% water content and the blue colour develops up to about 28% water contents. Using a solution of carotene in benzene and shaking it with sulfuric acid of different concentration, we found that a partition equilibrium is established between the acid and benzene phase. At about 84% H_2SO_4 and above all carotene passes into the acid, while below 72% all of it remains in the benzene or reverts into benzene solution if a stronger acid is diluted with water. At concentrations between 72 and 84% H_2SO_4, the carotene is distributed between the yellow benzene and the blue acid phase reversibly. It should be proved, however, whether the reverted yellow pigment is genuine carotene or a derivate therefrom. We also wish to investigate the partition equilibrium between these phases quantitatively. It is interesting to note that the upper and lower concentration limits of the sulfuric acid at both sides of this partition equilibrium approximate very closely to the composition for $H_2SO_4 \cdot H_2O$ and $H_2SO_4 \cdot 2H_2O$ respectively, although in view of the fact that carotenoids containing oxygen sometimes give the blue colour even with dilute acids, this agreement may prove to be a mere coincidence.

These blue solutions were subjected to an electric field strength of about 1 V/cm in the inverted V-tube apparatus. To stabilize the boundary between coloured solution and the underlying strong sulfuric acid, the former was slightly diluted with water or methanol. To our great surprise these blue derivates of the carotenes migrated to the anode. Their ionic mobilities were of the order of 10^{-4}, which may be regarded as normal in viscous sulfuric acid media. The fractions of the chromatogramm of oxydised carotene mentioned above were treated similarly and this time they too all migrated towards the anode! The cationic or anionic behaviour thus depends upon whether we work in aqueous acid solutions or in acidified isobutyl alcohol (or amyl alcohol). The 6th fraction (luteochrome) dissolved to a grayish-green colour, the 7th fraction (diepoxide) to greenish-blue while the others varied between brown and violet.

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[1] F. Körösy and Gy. Székely, Nature *168*, 77 (1951); Magyar Kémiai Folyóirat. *57*, 110 (1951).

[2] F. Körösy and Gy. Székely, Magyar Kémiai Folyóirat. *58*, 174 (1952).

[3] P. Karrer and E. Jucker, Helv. Chim. Acta *28*, 427, 470 (1945).

The anionic behaviour could be explained simply by assuming that sulfuric acid is added to the conjugated system and conveys an acidic character to the carotenoids. However, such a reaction should not involve the change of colour from yellow to blue, nor would it be easy to reconcile this view with the reversibility of the reaction by simple addition of water.

Nothing was known until now about the existence of an electrochemistry of carotenoids. Our experiments revealed not only its existence but also its rather complicated nature. We wish to extend these investigations to a larger number of well defined carotenoids in acids of different strength in different solvents, and we should be glad if specialists in carotenoid chemistry would join in this investigation. We hope to be able to investigate also the magnetic behaviour of the yellow, green and blue solutions.

I wish to express my gratitude to Prof. P. TUZSON for having made me acquainted with carotenoid chromatography and for his valuable advice concerning purification and identification of carotenoids. I am also greatly indebted to Prof. P. KARRER for his valuable and helpful correspondence.

F. KŐRÖSY

Délibáb ú. 28, Budapest, August 3, 1954.

Zusammenfassung

Carotinoide und Carotinoid-Epoxyde bilden mit verschiedenen Säuren und Salzen intensiv blau gefärbte Produkte, deren Natur unbekannt ist. Das elektrolytische Verhalten dieser Stoffe wird untersucht, und es wird festgestellt, dass die Wanderungsrichtung in hohem Mass davon abhängt, ob die Behandlung der Carotinoide mit Säuren in wässeriger oder in wasserfreier alkoholischer Lösung vorgenommen wurde.

The Identity of Vincamajoridine and Akuammine

Akuammine, $C_{22}H_{26}O_4N_2$, the main alkaloid in the seeds of *Picralima Klaineana*[1] and *P. nitida*[2] was recognized as a 5-hydroxy-N-methylindoline as a result of a study of its reactions and of its U.V. and I.R. spectra[2].

Independently, vincamajoridine, recently isolated from *Vinca major* L., was similarly characterized as a 5-hydroxy-N-methylindoline of the formula $C_{22}H_{26}O_4N_2$[3].

The descriptions of the two substances left little doubt in our minds that they were identical and a direct comparison has established that this is indeed the case. The specimen of vincamajoridine, separated chromatographically, was slightly more pure than that of akuammine. Both specimens crystallized from ethanol in short, slender, microscopic needles of identical appearance.

Heated together in capillaries in the same bath vincamajoridine had (uncorr.) m.p. 258–260° (decomp.), and akuammine had m.p. 255° (decomp.); a mixture had m.p. 255–256° (decomp.). The m.p. varied with the rate of heating but a mixture of the two specimens never melted lower than did the akuammine.

The colour reaction with ferric chloride was exhibited identically by the two specimens in respect of colour, intensity, and duration. Vincamajoridine has $[\alpha]_D$-104° ± 4° (pyridine)[1]; we now find that akuammine has $[\alpha]_D^{16}$-105·3° (c, 1·31 in pyridine).

The U.V. absorptions of akuammine[2] and vincamajoridine[1] are the same within experimental error and the complete correspondence of the I.R. spectra is quite remarkable. The curve has already been recorded[1]. Incidentally this agreement in spectra of specimens isolated from different plants, by two groups of workers, and taken on different instruments, shows that even apparently insignificant details are characteristic and signalize real properties of the molecule.

M. M. JANOT[3], J. LE MEN[3]
K. AGHORAMURTHY[4], and
R. ROBINSON[4]

Laboratory of Galenic Pharmacy, Faculty of Pharmacy, University of Paris, and Dyson Perrins Laboratory, Oxford University, June 3, 1955.

Résumé

Les auteurs démontrent que la vincamajoridine $C_{22}H_{26}O_4N_2$; F. 258; $(\alpha)_D$-104 ± 4° (py) récemment extraite de la Grande Pervenche (*Vinca major* L.) est identique à l'akuammine antérieurement découverte dans les graines d'une autre Apocynacée *Picralima nitida* (STAPF) T. et H. DURAND.

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[1] T. A. HENRY and T. M. SHARP, J. Chem. Soc. *1927*, 1950. – T. A. HENRY, J. Chem. Soc. *1932*, 2759.

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[1] M. M. JANOT and J. LE MEN, C. r. Acad. Sci. *240*, 909 (9155).

[2] M. RAYMOND-HAMET, C. r. Acad. Sci. *230*, 1183 (1950).

[3] Laboratory of Galenic Pharmacy, Faculty of Pharmacy, University of Paris.

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Oriented Crystallization of Inorganic Salts in Collagen

In an attempt to study in detail the reported "addition compounds" of collagen with alkali[1], the authors observed some interesting phenomena which are reported here. All the experiments described below were performed with kangaroo tail tendon.

A fibre of collagen about 1 mm diameter was kept under tension in 0·25 N sodium hydroxide for about 15 h and was then dried. An x-ray photograph of this specimen is reproduced in Figure 1. In addition to the collagen pattern, the figure exhibits a number of spots on definite layer lines, suggestive of the existence of well crystallized material all oriented parallel to the fibre axis. The spots (excluding those due to collagen) could be indexed on the basis of an orthorhombic unit cell with a = 5·25 A, b (fibre axis) = 6·45 A, c = 10·7 A. On repeating the experiment a second pattern was obtained, which again corresponded to a well crystallized material, oriented with one of its axes along the fibre, but whose unit cell was monoclinic with a = 20·4 A, b (fibre axis) = 3·5 A, c = 10·3 A, β = 106°. The positions of the spots closest to the direct beam in the first pattern agreed with those reported earlier[1], but those in the second pattern were quite different. The sharpness of the reflections and also the fact that they could be observed right up to Θ = 90°, indicated that they cannot be ascribed to an

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[1] G. L. CLARK and A. SCHAAD, Radiology *27*, 339 (1936).