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.

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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.

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

[2] M. F. MILLSON, R. ROBINSON, and A. F. THOMAS, Exper. *9*, 89 (1953).

[3] M. M. JANOT and J. LE MEN, C. r. Acad. Sci. *240*, 909 (1955).

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 $[\alpha]_D^{15}$-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.

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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.

[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).

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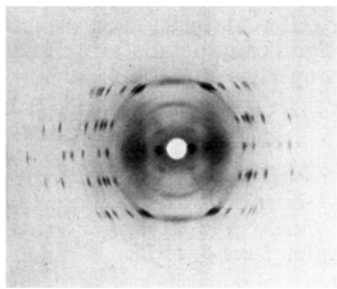
Oriented Crystallization of Inorganic Salts in Collagen

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

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

addition compound of collagen. A search of the literature showed that the unit cells of the two patterns agreed closely with those of sodium carbonate monohydrate (Na_2CO_3, H_2O) and sodium sesqui-carbonate (Na_2CO_3, $NaHCO_3$, $2H_2O$) respectively[1]. Thus, it was established that no addition compounds were formed, but that the phenomenon was one of oriented crystallization. Similarly, by keeping collagen in a solution of potassium hydroxide exposed to air, oriented crystallization of $KHCO_3$ has been achieved.



X-ray pattern of collagen in which Na_2CO_3,H_2O has crystallized with its *b*-axis parallel to the fibre axis, taken with a rotation camera 3 cm radius with the fibre axis kept parallel to the axis of rotation of the camera, using CuKα radiation. Photograph reduced by ½ in reproduction.

The specimens containing the crystallized salts are extremely stable, if kept dry, and the x-ray pattern is reproducible even after a month. However, if the specimen is dipped in distilled water for a few seconds and taken out, the salt pattern disappears.

If collagen is kept in freshly prepared sodium hydroxide solution in a full well-stoppered bottle, the above phenomenon was not found, indicating that carbonate produced by contact with air was necessary. So also, if the collagen fibre was kept in a strong solution of sodium carbonate in water, the salt did not enter and crystallize in the collagen. However, if the solution was made alkaline by adding a small amount of sodium hydroxide, then the specimen on drying contained oriented crystals of sodium carbonate. In fact, oriented crystallization of $NaHCO_3$ and Na_2SO_4 has also been achieved by using alkaline solutions of these salts.

It has not yet been possible to explain why the alkali is required. It apparently helps by swelling the collagen and thus enabling the salt to get in. An interesting fact observed with regard to the various salts which crystallized in collagen is that the unit cell dimension along the fibre axis always had a value between 3·2 and 3·75 A or a multiple of this.

A fuller discussion of the experiments will be published in the Proceedings of the Indian Academy of Sciences.

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Zusammenfassung

Wenn gestreckte Kollagenfasern in einer alkalischen Lösung gewisser anorganischer Salze gelassen und dann getrocknet werden, so kristallisieren die Salze mit einer ihrer Achsen in paralleler Orientierung zur Faserachse. Die Periode parallel dieser Achse liegt zwischen 3,2 und 3,75 Å oder einem Vielfachen dieser Werte.

[1] R.W. G. WYCKOFF, *Crystal Structures* (Interscience Publishers 1951), Vol. II, Ch. X, p. 3. – *Structure Reports*, N.V.A. Oosthoek's Uitgevers Mij, Vol. 12, p. 238 (1949).

The Effect of Vitamin B_{12} on the Coenzyme A Content of Normal Strains of *Escherichia coli*[1]

In an earlier investigation, the author observed that the addition of vitamin B_{12} to cultures of "wild" strains of *Escherichia coli* caused an inhibition of the synthesis of free pantothenic acid. It was the author's object in the present report to investigate whether there is any relationship between Vitamin B_{12} and coenzyme A in such strains of *E. coli* as give a positive reaction in the above-mentioned respect. BOXER and his coworkers[2] have previously investigated the relation between vitamin B_{12} and coenzyme A in rat liver cells. They found a definite interrelationship; the coenzyme A content in the liver cells of rats on a vitamin B_{12} deficient diet was considerably higher than the corresponding value of rats receiving additional vitamin B_{12} in the diet.

In his experiments, the author at first isolated from normal human faeces some strains of "wild" *E. coli* and tested the effect of vitamin B_{12} on the pantothenate synthesising ability of these strains. The methods used in these experiments are described in an earlier paper (JÄNNES[3]). The author only modified the culture medium used in this work in the following way: the earlier medium used contained as source of organic nitrogen seven amino acids: Tryptophane, proline, arginine, histidine, glycine, glutamic acid and asparagine. Instead of these, a solution of vitamin-free casamino acids (Difco) with addition of tryptophane and asparagine was used.

The composition of the medium was as follows: K_2HPO_4, 10 g; KH_2PO_4, 4·5 g; sodium citrate 0·75 g; $(NH_4)_2SO_4$, 1·5 g; $MgSO_4 \cdot 7\ H_2O$, 0·15 g; glucose, 15 g; l-asparagine, 4 g; vitamin-free casamino acids (10% solution), 30 ml; dl-tryptophane, 100 mg; distilled water to make 1000 ml. The pH was adjusted to 7·0.

The procedure was as follows: immediately after the preliminary experiments which were mentioned above, strains whose free pantothenate synthesis was inhibited by added vitamin B_{12} were cultivated on stab agar cultures and kept in a refrigerator. On the following day, the effect of vitamin B_{12} on the coenzyme A content of these strains was examined. It was noted that three of the seven investigated strains showed definite inhibition.

For the assay of coenzyme A, the strain was cultivated in two 2 litre ERLENMEYER bottles, with and without addition of vitamin B_{12}. The same medium as in the preliminary experiment was used. The growth of the bacteria was not in any way affected by the addition of vitamin B_{12}. After 24 h growth the cells were harvested with a Sharpless supercentrifuge and dried in a thin layer over phosphorous pentoxide in a vacuum exsiccator. It was found necessary that the conditions during the drying procedure were absolutely the same for both samples.

Exactly 100 mg of dried bacteria was weighed from each sample and suspended in 3 ml of water. Both suspensions were than boiled in a gaseous flame for exactly the same time.

After centrifugation, the coenzyme A content of the supernatants was determined by the method of LIPMANN and KAPLAN[4]. The "transacetylase enzyme" was pre-

[1] This work has been aided by grants from Emil Aaltonen Foundation and Sigrid Juselius Stiftelse.

[2] G. F. BOXER, W. H. OTT, and O. E. SHONK, Arch. Biochem. Biophys. *47*, 474 (1953).

[3] L. JÄNNES, Ann. Acad. Sci. Fenn. Suppl. *61*, 39 (1954); Exper. *10*, 31 (1954).

[4] N. O. KAPLAN and F. LIPMANN, J. Biol. Chem. *174*, 37 (1948).