

found that more than one-third of the isolates (moulds and bacteria) had inhibiting effects on the *annosus* mycelium *in vitro*, one of them being *Trichoderma viride*, which was inhibiting at 22°C, but was itself overgrown by *P. annosus* at 12°C.

In the present investigation, a number of actinomycetes from Danish forest soil (with and without the occurrence of the fruiting bodies of *Polyporus annosus*) was isolated. Nearly half of the isolates showed antagonistic effects on the *annosus* mycelium *in vitro*; one of these isolated actinomycetes was used in the following experiments.

A series of bowls with sterile garden soil (air-dried, with the addition of sufficient water to obtain a good crumb condition, pH after autoclaving, 7.0) was inoculated with suspensions of spores of the actinomycete (*Streptomyces* sp.) and subsequently incubated for 7 days at 22°C. A control series of bowls with sterile but uninoculated soil was run. In all bowls—in both series—a young agar culture of *annosus* mycelium grown on a slide was then placed after the incubation period, with the mycelium side downwards on the surface of the soil. After a few days, the mycelium in the control series began to spread over the free surface of the soil, but in the series inoculated with *Streptomyces* sp. little or no growth of *Polyporus annosus* from the edges of the slides was observed.

The experiments were repeated, with the difference that instead of slide cultures, 10 mm diameter agar disks cut from Petri dish cultures of *P. annosus* were used. The inhibition in these experiments was very marked.

It is not possible to make any conclusion from the experiments described here as to the more complex conditions in nature, but it may be justifiable to advance the theory that actinomycetes in natural soils may be partially responsible for the non-occurrence of *Polyporus annosus* in some areas in the forests. In this connection it is reasonable to ask if it would not be possible to stimulate the flora of actinomycetes in forest soil or give it a favourable trend by the addition of suitable green manures.

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Zusammenfassung

Eine Reihe von Actinomyceten aus dänischen Waldböden wurden auf antagonistische Wirkung gegen den Wurzelfäulnispilz, *Polyporus annosus* Fr., geprüft. Einer der isolierten Organismen wirkte auch in sterilem Boden hemmend auf das Myzel von *Polyporus annosus*. Die Möglichkeit wird erwähnt, den Wurzelfäulnispilz im infizierten Boden durch Förderung des Actinomycetenwachstums mit Gründüngung zu beeinflussen.

Studies on the Ascorbic Acid and Carotene Content in Leaves as a Common Characteristic of Botanically Related Species

It has been observed that there are plant families with a high ascorbic acid content, while other families

are markedly poor in this vitamin. Thus, a high anti-scorbutic activity has been noted in leaves of Cruciferae, while on the other hand Compositae were found to contain relatively small amounts of vitamin C¹. No study has so far been undertaken of the carotene content in leaves as a common property of plants belonging to the same family, but it has been observed that the carotene and chlorophyll content of higher plants run parallel with the ascorbic acid content². This has been explained through the possibility of a certain functional correlation of vitamin C and these pigments³.

The present communication concerns the results of an examination of the ascorbic acid and carotene content in leaves, which was carried out on a large scale as a part of systematic studies on the vitamin value of edible wild plants growing in Yugoslavia. This work includes the examination of 74 plant species from 26 families, the results being tabulated and made known in two previous publications⁴. Altogether 503 analyses of ascorbic acid and 200 carotene determinations have been carried out. However, the present study is limited to 7 plant families only; the number of species investigated of the remaining 19 families is too small to permit any general conclusions. Ascorbic acid was determined by titration of the metaphosphoric acid extract with 2,6-dichlorophenol-indophenol, according to a standard procedure⁵. Carotene was determined by the method described by CURTIS⁶, based on the chromatographic adsorption of the petroleum ether extract on a column of soluble starch. It was calculated as beta-carotene, which accounts for about 90% of the total carotene in fresh leaves⁷. Both determinations were carried out with fresh samples immediately after gathering.

In Table I the results obtained by leaf analysis of the 7 plant families are summarized. Since a single species has been analyzed 4–12 times for vitamin C and 2–6 times for the carotene content, only the average values are given in the Table.

As to the ascorbic acid content of Cruciferae and Compositae, Table I confirms the values reported in the literature cited above. The Cruciferae examined were rich (average 182 mg%), and Compositae relatively poor (average 37 mg%) in this vitamin. According to our results it seems probable that there are also families distinguished by a relatively high carotene content (Leguminosae and Labiatae). However, in general, the amounts of ascorbic acid and carotene usually vary considerably within the same family, the differences between various species being sometimes very marked. On the basis of the data obtained, it will be hardly possible to classify categorically each plant family as more or less rich in the ascorbic acid or carotene content. We often found species belonging to one family "rich" in vitamin C whose leaves contained much smaller quantities of ascorbic acid than did the average of the family. In the leaves only of such plants we also often found a

¹ T. HIRAKA, Seiri Seitai 1, 61 (1947). – A. SEYBOLD and H. MEHNER, Sitz.-Ber. Heidelberg. Akad. Wiss. math.-naturw. Klasse 1948, 213.

² J. C. DRUMMOND, H. J. CHANNON, and K. H. COWARD, Biochem. J. 19, 1047 (1925). – H. EULER, V. DEMOLE, and P. KARRER, Z. physiol. Chem. 183, 43 (1932). – O. A. BESSEY and C. G. KING, J. biol. Chem. 103, 687 (1933).

³ A. GIROUD and A. R. RATSIMAMANGA, Acide ascorbique vitamine C (Paris 1942), p. 123.

⁴ Lj. GRLIČ, Acta pharm. Jugosl. 2, 112 (1952); 4, 115 (1954).

⁵ Methods of Vitamin Assay, Association of Vitamin Chemist Inc., (2nd ed., New York 1951), p. 76.

⁶ O. F. CURTIS, Plant Physiol. 17, 133 (1942).

⁷ A. HUZITA and M. AZISAKA, Biochem. Z. 308, 430 (1941).

Table I

Plant family	Number of species investigated	Number of samples analyzed		Values obtained in mg%			
		Ascorbic acid	Carotene	Ascorbic acid		Carotene	
				Range	Average	Range	Average
Polygonaceae	8	53	20	60–163	105	4.9–15.6	9.7
Chenopodiaceae	4	25	11	29–153	95	2.4–12.8	9.5
Cruciferae	11	70	24	92–334	182	6.1–13.8	8.5
Leguminosae	5	32	16	67–175	129	9.2–22.0	13.9
Umbelliferae	5	29	13	20– 99	76	2.1–13.9	7.5
Labiatae	3	20	7	41– 83	56	10.9–21.5	15.2
Compositae	10	74	29	19– 51	37	5.1–12.5	8.5

decreased carotene content, so that the amounts of the two constituents were parallel, and the ascorbic acid/carotene ratio (a/c) remained nearly the same as in other plants of the family. A striking example showing the approximate constancy of the a/c ratio were plants of xerophytic structure. The leaves of *Salsola Kali* L., for example, were proportionally much poorer in both vitamins than the other Chenopodiaceae examined.

In Table II the calculated ratio of the ascorbic acid/carotene content (a/c), for the plant families examined, is shown.

Table II

Plant family	The calculated a/c ratio	
	Range of the species examined	Average of the family
Polygonaceae	9.2–12.2	10.8
Chenopodiaceae	8.8–12.1	10.0
Cruciferae	11.8–34.5	21.4
Leguminosae	7.3–12.3	9.3
Umbelliferae	6.3–16.5	10.1
Labiatae	3.3– 3.9	3.7
Compositae	2.6– 6.5	4.4

In Table II a certain tendency to constancy in the a/c ratio can be observed. By comparing the data of the two tables, the a/c ratio seems to be less variable and more characteristic of a given family than the ascorbic acid or carotene content itself. While the amounts of ascorbic acid and carotene in various Polygonaceae, for example, varied quite considerably (from 60 to 163 and from 4.9 to 15.6, resp.), the a/c ratio in various species of this family remained rather constant (from 9.2 to 12.2). Such is the case also with Chenopodiaceae, Leguminosae, Labiatae and Compositae. Cruciferae and Umbelliferae showed, however, a much wider range in this ratio. It is interesting to note that 4 of 7 families examined have an average a/c ratio of nearly 10.

Since the vitamin content of plant tissues varies within species between very wide limits and is influenced by many factors (soil, shade, moisture, season, stage of growth etc.), it is obvious that no conclusion concerning the exact quantitative correlation between ascorbic acid and carotene in plants can be drawn here. However, from the data of our tables it is readily apparent that the ascorbic acid/carotene ratio shows within plant families a greater tendency towards constancy than do the concentrations of these constituents themselves. This fact seems to support the view that there may be a closely

connected functional interrelationship between these two constituents in plants.

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Résumé

Les résultats des dosages parallèles d'acide ascorbique et de carotène dans les feuilles des espèces appartenant à 7 familles ont démontré une tendance à la stabilité de la proportion acide ascorbique/carotène chez les plantes apparentées. La stabilité de cette proportion chez les espèces d'une seule famille est plus marquée que celle de la teneur même des deux substances. Ce fait semble pouvoir confirmer l'idée d'une corrélation étroite dans l'action des deux substances en question.

Über die Einwirkung des Schilddrüsenhormons auf die Ossifikation

Hinsichtlich der Frage von Beziehungen der Schilddrüsenfunktion zum Kalkstoffwechsel, insbesondere über eine etwaige Einwirkung des Schilddrüsenhormons auf den Kalzifikationsvorgang bei der Knochenbildung, gehen die Ergebnisse bisheriger Untersuchungen weit auseinander: SCHULZE¹ entwickelt die Vorstellung, dass nur die Derivate des Ektoderms und des Entoderms auf den Thyroxinreiz anzusprechen vermögen, KELLER², FOX und IRVING³ und KALTENBACH⁴ haben in ihren Experimenten Hinweise auf eine Förderung der Ossifikation durch das Schilddrüsenhormon erhalten.

Für die experimentelle Klärung dieses Problems ergeben sich folgende Fragestellungen: 1. Sind bei einer durch Verabreichung von Schilddrüsenhormon ausgelösten, vorzeitigen Metamorphose bezüglich der Kalk-einlagerung im Stützgewebe Veränderungen festzustellen, die denjenigen bei der experimentell unbeeinflussten Verwandlung entsprechen? 2. Sind bei einer lokalen Thyroxineinwirkung örtlich begrenzt Veränderungen in bezug auf die Kalkeinlagerung festzustellen?

Larven von *Salamandra salamandra quadrivirgata* Dürigen wurden im geburtsbereiten Stadium dem Ute-

¹ W. SCHULZE, Roux' Arch. 101, 338 (1924).
² R. KELLER, Rev. Suisse Zool. 53, 329 (1946).
³ E. FOX und J. T. IRVING, S. Afr. J. med. Sci. 15, 11 (1950).
⁴ J. C. KALTENBACH, J. exp. Zool. 122, 21 (1953).