Phytochemistry, 1969, Vol. 8, pp. 1311 to 1312. Pergamon Press. Printed in England.

SHORT COMMUNICATION

THE OCCURRENCE OF ZEINOXANTHIN IN ALFALFA

A. L. LIVINGSTON and R. E. KNOWLES

Western Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Albany, California 94710, U.S.A.

(*Received* 28 *December* 1968)

Abstract—A monohydroxycarotenol has been isolated from alfalfa and shown to be zeinoxanthin.

A PREVIOUS study at this laboratory[1] showed the five main xanthophylls of fresh alfalfa to be lutein, violaxanthin, neoxanthin, zeaxanthin and cryptoxanthin. In addition, seven minor xanthophylls were found to be present. Two of these were tentatively identified as monohydroxy-α-carotene-like. Moster, Quackenbush and Porter[2] examined the carotenoids of corn seedlings and also identified one of the xanthophylls as monohydroxy-α-carotene-like. Subsequently, Petzold and Quackenbush[3] isolated and characterized a monohydroxy carotenol from corn gluten which they designated as zeinoxanthin.

In the course of our continuing investigation of the carotenoids of alfalfa we have identified zeinoxanthin as one of the minor carotenoids of alfalfa. Comparison of the spectra and co-chromatography of the apparent zeinoxanthin from alfalfa and an authentic specimen prepared from corn gluten meal confirmed the identity.

EXPERIMENTAL

Ground freeze-dried alfalfa *Medicago sativa* (Leguminosae) (1 kg) was extracted for 48 hr at room temperature with (15 l.) hexane–acetone (7:3). The extract was saponified overnight with 60 ml of a saturated solution of KOH in methanol. Following washing with water to remove the alkali and polar solvent, the

TABLE 1. SPECTRAL ABSORPTION OF ZEINOXANTHIN

Source of zeinoxanthin	Solvent	λ_{max} (in nm)		
Alfalfa	Hexane	422	445	473
Corn gluten	Hexane	421	445	473
Alfalfa	Chloroform	434	456	485
Corn gluten	Chloroform	434	456	485
Alfalfa	Carbon disulfide	449	474	505
Corn gluten	Carbon disulfide	449	474	505

[1] E. M. BICKOFF, A. L. LIVINGSTON, G. F. BAILEY and C. R. THOMPSON, *J. Agr. Food Chem.* **2**, 563 (1954).
[2] J. B. MOSTER, F. W. QUACKENBUSH and J. W. PORTER, *Arch. Biochem. Biophys.* **38**, 287 (1952).
[3] E. N. PETZOLD and F. W. QUACKENBUSH, *Arch. Biochem. Biophys.* **86**, 163 (1960).

extract was dried over anhydrous Na_2SO_4. The saponified extract was then submitted to column chromatography on magnesium oxide–Hyflo Supercel (1:1, w/w). The zone just above the carotene and below the dihydroxy xanthophylls was separated and the pigment eluted. The fraction was concentrated to remove the acetone and rechromatographed on lime–Hyflo Supercel (1:1, w/w). The column was developed with ethyl ether–hexane (1:4, v/v) as described by Petzold and Quackenbush. The band corresponding to zeinoxanthin was collected and repurified by TLC on lime–silica gel G (4:1, w/w), employing benzene–butanol (100:2) as the developing solvent. Visible spectra in hexane, $CHCl_3$ and CS_2 (Table 1) were found to be identical with those of a sample of zeinoxanthin prepared from corn gluten meal. Tests for allylic hydroxyl groups, performed by treating a $CHCl_3$ solution of the respective xanthophyll with anhydrous HCl gas in $CHCl_3$, were negative for the alfalfa and corn gluten samples but positive for a sample of 4-hydroxy-α-carotene. Co-chromatography of the sample prepared from alfalfa with the known specimen on columns of lime–celite, sucrose–celite and of Microcel C, and by TLC on lime-silica gel G. confirmed the identity (Table 2).

TABLE 2. R_f VALUES OF ZEINOXANTHIN ISOLATED FROM ALFALFA AND FROM CORN GLUTEN AND CHROMATOGRAPHED SINGLY AND IN ADMIXTURE ON TLC ADSORBENT

Adsorbent	Developing solvent	R_f
Lime–silica gel G (4:1)	Hexane–acetone (98:2)	0·27
Lime–silica gel G (4:1)	Benzene–butanol (98:2)	0·48
Lime–silica gel G (6:1)	Hexane–acetone (98:2)	0·47
Lime–silica gel G (6:1)	Benzene–butanol (98:2)	0·65