

to the final conclusion that the deamination reaction should occur at the first step of the ethanolamine decomposition as shown in scheme (b). The enzyme catalyzing the deamination reaction is named ethanolamine deaminase.

*Institute of Food Microbiology,  
University of Chiba,  
Okubo, Narashino, Chiba-ken.*

Komei Miyaki (宮木高明)  
Makoto Hayashi (林 誠)  
Tsutomu Unemoto (畝本 力)

November 17, 1958

UDC 547.979:582.932

### Carotenoids of the Flowers of *Osmanthus fragrans*

On the carotenoids of the flowers of *Osmanthus fragrans* LOUR. var. *aurantiacus* MAKINO, Ishiguro and co-workers isolated all-*trans*  $\beta$ -carotene during the course of their study on the aromatic component, osmane.<sup>1)</sup> It appeared of interest to investigate this problem further, because, from their comparatively yellowish color and from the biogenetic standpoint, the presence of usual carotenoids other than  $\beta$ -carotene and that of these epoxides or furanoid oxides was anticipated.<sup>2,3)</sup> For our purpose, every precaution was taken to avoid the effect of light, heat, and air oxidation. All operations were carried out in a dark room, at room temperature, at the highest and *in vacuo* when necessary. The extraction of pigments from the fresh flowers was conducted by the usual method<sup>4)</sup> immediately after their collection. After the concentrated petroleum ether extracts were developed on calcium hydroxide column (5×40 cm.; developer, hexane), the following chromatograms were obtained.

Width of zone (mm.)	
69	Four yellow and one pink zones : unidentified
43	Colorless interzone
24	Orange, all- <i>trans</i> $\beta$ -carotene ( $\lambda_{\max}^{\text{Hexane}}$ 478, 450 m $\mu$ )
79	Interzone
8	Pale yellow, neo- $\beta$ -carotene B ( $\lambda_{\max}^{\text{Hexane}}$ 474, 444 m $\mu$ )
63	Interzone
18	Yellow, all- <i>trans</i> $\alpha$ -carotene ( $\lambda_{\max}^{\text{Hexane}}$ 475, 445 m $\mu$ )
15	Interzone
6	Pale yellow, traces, unidentified

Up to the present, the expected epoxides or furanoid oxides have not been isolated yet, but besides all-*trans*  $\beta$ -carotene reported previously, all-*trans*  $\alpha$ -carotene, neo- $\beta$ -carotene B, and five other unidentified pigments were separated. It was also confirmed that such stereoisomer of  $\beta$ -carotene can exist in nature,<sup>3,5)</sup> though several isomers were obtained and studied extensively *in vitro*.<sup>6)</sup> The structure of neo- $\beta$ -carotene B was assigned as 9,13'-di-*cis*- $\beta$ -carotene.<sup>7)</sup> If this stereoisomer came secondarily from

- 1) T. Ishiguro, N. Koga, K. Nara : *Yakugaku Zasshi*, **77**, 566, 681(1957).
- 2) K. Tsukida, L. Zechmeister : *Arch. Biochem.*, **74**, 408(1958).
- 3) L. Cholnoky, *et al.* : *Acta Chim. Acad. Sci. Hung.*, **6**, 143(1955).
- 4) L. Zechmeister, J. H. Pinckard : *J. Am. Chem. Soc.*, **69**, 1930(1947).
- 5) J. W. Porter, F. P. Zscheile : *Arch. Biochem.*, **10**, 537(1946); J. C. Sadana, *et al.* : *J. Sci. Ind. Research*, **6**, 47(1947); **7**, 172(1948); *Indian J. Med. Research*, **37**, 193(1949); *Biochem. J.*, **44**, 401(1949); E. M. Bickoff, C. R. Thompson : *J. Assoc. Offic. Agr. Chemists*, **32**, 775(1949); **34**, 460(1951).
- 6) L. Zechmeister : *Chem. Revs.*, **34**, 314(1944); *Experientia*, **10**, 1(1954).
- 7) K. Lunde, L. Zechmeister : *J. Am. Chem. Soc.*, **77**, 1647(1955).

the corresponding all-*trans* compound during the course of the present experiment, another main isomer, neo-U, should also appear more or less on the same column, but any trace of such a compound was not observed in these experiments. The photo-metrically established ratio of the contents was approximately 80:2:18 for all-*trans*  $\beta$ -carotene:neo- $\beta$ -carotene B:all-*trans*  $\alpha$ -carotene. Each pigment was identified by comparing their spectroscopic data, m.p., etc., with those of the pure samples which were prepared by one of us during the collaborative study with Prof. L. Zechmeister, and by studying the spectral characteristics of their stereoisomeric mixtures after iodine treatment. Neo-B isomer was identified further by mixed chromatographic test with the pure substance which was derived from the corresponding all-*trans* compound, and also by its conversion to the all-*trans* compound by iodine treatment followed by mixed chromatography.

Kobe Women's College of Pharmacy  
Motoyama-cho, Higashinada-ku,  
Kobe.

Nobuo Suzuki (鈴木信夫)  
Kiyoshi Tsukida (月甲 潔)

November 19, 1958

UDC 547.917:582.76

### The Constituents of Pueraria Root

The Pueraria root (*Pueraria Thunbergiana* BENTH. (Japanese origin); *P. pseudo-hirsuta* TANG et WANG, and *P. Thomsonii* BENTH. (Chinese origin))<sup>1)</sup> is known in traditional Chinese medicine as an important medicament whose effect would suggest that it may contain some antispasmodic active principle.

Although it is well known that the root contains a large amount of starch and therefore it is productively used as its source, the existence of any other special constituents in the root has not been reported as yet. We have investigated the constituents of Pueraria root to see if any curative principle could be isolated from it.

Pueraria roots of Japanese and Chinese origin were extracted with MeOH and the extracts were separated by means of lead acetate method.

The fraction precipitated with basic lead acetate was collected (40~50 g. from 1 kg. of the dried, commercial crude drug; 40 g. from 2 kg. of fresh Pueraria root) and was separated by means of column chromatography using  $Al_2O_3$  as an adsorbent and water-saturated BuOH as the original developing solvent. The chromatogram consisted of one non-fluorescent and nine violet-bluish fluorescent bands which were visualized under ultraviolet irradiation (bands *a* to *j*, from bottom to top in the chromatogram; band *c* gives no fluorescence).

The developing solvent was changed to a mixture of BuOH and pyridine (10:1) after the band *e* was removed, and again altered to a mixture of aq. BuOH and AcOH(10:1) after elution of band *g* was completed. The fractions obtained by chromatography were chemically examined (Table I).

The properties of crystals obtained from the fraction *b* indicated that it would be an isoflavone derivative and its identification with daidzein, the aglycone of daidzin isolated first from soybeans,<sup>2,3)</sup> was established by a mixed fusion of its dimethyl ether with the synthetic sample.

1) Hu Hsien Su: "Handbook of Useful Plants" (經濟植物手冊)(in Chinese), Vol. II-1, 780(1957).