immunochemical complex with antibody embedded in agar gel. Double diffusion of antigen/antibody solns was performed according to ref. [10] in 1.5% Ionagar. Qualitative aminopeptidase activities on the immunoprecipitates were detected by incubating the dried plates for 2 hr in 100 ml of 0.2 M Pi buffer, pH 4.4, containing 20 mg of L-leucyl-beta-naphthylamide-HCl as substrate, and 25 mg of Black Salt K. This technique, described in ref. [11], is particularly useful in identification procedures within a mixture of enzymes or other proteins. The immune sera were prepared by injections in 3 rabbits of proteins in appropriate fractions, according to the standard procedure of Antibodies Incorporated, Davis, California. Specific aminopeptidase activity was determined by the method of ref. [12]. Activities are expressed as katals per mg protein; a unit is defined as one mol of substrate hydrolyzed per sec at 30°.

Acknowledgements—The determinations of sp. act. of aminopeptidases and discussions of the subject matter with Dr Juhani Mikola, Department of Biology, University of Jyväskylä, Jyväskylä 10, Finland, and the line drawing of Nancy Meadow, are gratefully acknowledged.

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SPECIFICITY OF ENZYME SYSTEM PRODUCING C_6-ALDEHYDE IN *THEA* AND *FARFUGIUM* CHLOROPLASTS

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(*Received* 31 *May* 1977)

Key Word Index—*Thea sinensis*, Theaceae; *Farfugium japonicum*, Compositae; isolated chloroplasts; lipoxygenase; *cis*-3-hexenal; *n*-hexanal; *cis*-3, *cis*-6-dienoic acids (C_8–C_{12}); substrate specificity

Recently it was confirmed that linolenic acid is split into C_6-aldehyde, *cis*-3-hexenal, and C_{12}-oxo-acid, 12-oxo-*cis*-9-dodecenoic acid, by an enzyme system in chloroplasts of tea leaves, using linolenic acid-[^{14}C] [1, 2]. The enzyme system in tea chloroplasts catalyzes the oxidative splitting of C_{18}-unsaturated fatty acids through addition of oxygen to the double bond at C-12 and produces C_6-aldehydes. The structural requirement for substrates is the presence of a free carboxyl group and a *cis*-1, *cis*-4-pentadiene system including the double bond at C-12 [3]. *Farfugium japonicum* chloroplasts have also given a similar result to that of tea chloroplasts [4].

This report describes the substrate specificity of an enzyme system producing C_6-aldehydes for a series of synthesized *cis*-3, *cis*-6-dienoic acids in which the chain length varies from C_8 to C_{12} [5].

As shown in Table 1, when *cis*-3, *cis*-6-nonadienoic acid was used as a substrate, 40 and 67% of C_6-aldehyde (*cis*-3-hexenal) were produced by tea and *F. japonicum* chloroplasts, respectively, compared to *n*-hexanal formation from linoleic acid. *cis*-3, *cis*-6-Dodecadienoic acid gave 21 and 11% of C_6-aldehyde (*n*-hexanal). Neither acid (C_9 and C_{12}) gave any other volatile aldehydes (e.g. C_9-aldehydes). The other acids (C_8, C_{10} and C_{11}) were poor substrates or gave no expected aldehydes. On the other hand, these *cis*-3, *cis*-6-dienoic acids (C_8–C_{12}) did not act as substrates for soybean lipoxygenase.

Table 1. Substrate specificity of the enzyme system producing C_6-aldehydes

Substrate	Relative activity (%)		Product
	Tea	*F. japonicum*	
cis-3, *cis*-6-dodecadienoic acid	21	11	*n*-hexanal
cis-3, *cis*-6-undecadienoic acid	0	0	
cis-3, *cis*-6-decadienoic acid	0	0	
cis-3, *cis*-6-nonadienoic acid	40	67	*cis*-hexenal
cis-3, *cis*-6-octadienoic acid	0	<3	

Activities are expressed relative in mole number to *n*-hexanal obtained with linoleic acid.

It is concluded that *cis*-3, *cis*-6-nonadienoic acid and *cis*-3, *cis*-6-dodecadienoic acid, which have a double bond of *cis*-1, *cis*-4-pentadiene system at the 6th carbon atom counting from the terminal methyl group, act as a substrate for the enzyme system producing C_6-aldehydes in chloroplasts, though they have a smaller number of carbon atoms than linolenic and linoleic acids.

EXPERIMENTAL

Materials. Fr. leaves of *Thea sinensis* 'Yabukita' and *F. japonicum* Kitamura were used. A series of *cis*-3, *cis*-6-dienoic acids (C_8–C_{12}) were synthesized through unequivocal routes and were proved to be of 99% purity [5].

Enzyme assay. The activity of enzyme system producing C_6-aldehydes was determined as in refs. [3, 4] except that the incubation lasted for 10 min at 35° and volatile aldehydes produced from *cis*-3, *cis*-6-dienoic acids were analyzed by GLC. Soybean lipoxygenase (Miles Lab., Inc.) was assayed with 50 mM pyrophosphate buffer (pH 8.5) as in ref. [6].

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Phytochemistry, 1978, Vol 17, pp 549–550 Pergamon Press Printed in England

3-(3-HYDROXYMETHYLPHENYL)-L-ALANINE AND RELATED AMINO ACIDS IN *IRIS* SPECIES

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(*Received* 19 *August* 1977)

Key Word Index—*Iris sibirica*; *Iris sanguinea*; Iridaceae, 3-(3-hydroxymethylphenyl)-L-alanine; 3-(3-carboxyphenyl) alanine; 3′-carboxyphenylglycine; non-protein amino acids.

The free amino acids have been studied in leaves of *Iris sanguinea* Donn (syn. *I. sanguinea* Hornemann, *I. orientalis* Thunberg) and in seeds of *I. sibirica* L. 3-(3-Hydroxymethylphenyl)-L-alanine (**1**), one of the major free amino acids, has been isolated and 3-(3-carboxyphenyl)alanine (**2**) has been identified chromatographically in both species. In addition, the presence of 3′-carboxyphenylglycine (**3**) in *I. sanguinea*, but not in *I. sibirica*, has been established chromatographically. The identities of the samples were established by comparison of R_f values and PC, UV, PMR and mass spectra with those for the synthetic DL-compound [1].

For the isolate from *I. sanguinea*, the rotation at the D-line was found to be −25.5° in H_2O and −5.5° in 1N HCl. This establishes the L-configuration according to the Clough–Lutz–Jirgensons rule [2]. The molecular rotation values (−50 and −10.7°) are negative as expected and very near to those for L-phenylalanine (−57 and −7.4°) [3]. Similar rotation values were found for the sample from *I. sibirica*. Further proof of the L-configuration was obtained by determination of the CD-curve in HCl. A peak at about 217 nm with $\Delta\varepsilon$ + 3.9 was found for both samples. This peak is similar in sign and magnitude to that of L-phenylalanine ($\Delta\varepsilon$ + 3.61 at 216 nm) [4].

The R_f values of **1** in butanol–acetic acid–water and in phenol–ammonia–water are identical to those for γ-aminobutyric acid. Since the latter compound is universal in its occurrence in plants, **1** may easily escape detection in PC surveys. However, high voltage electrophoresis at pH 3.6 and PC in 2.4-lutidine–water easily distinguish between the two amino acids, and—as mentioned above—separation can easily be achieved by adsorption of the aromatic amino acid onto carbon.

The fraction of amino acids from *I. sanguinea* contained both **2** and **3** as established by PC whereas in the same fraction from *I. sibirica* only **2** was found.

Both *I. sanguinea* and *I. sibirica* belong to the subsection *Apogon*, subgenus *Limniris* of the genus *Iris* [5]. Previously **2** and **3** have been identified in *I.* × *hollandica* Bergm. cv Wedgwood and in *I.* × *hollandica* Bergm. cv Prof. Blauw [6–8]. These hybrids belong to the subgenus *Xiphium* [5]. Investigations are presently being performed to established the distribution of **1**, **2** and **3** within the Iridaceae.

1 has previously been isolated from *Caesalpinia tinctoria* (Leguminosae) but the configuration was not determined since no rotation measurements could be performed on the coloured isolate. In the same species was found 3-(3-hydroxymethyl-4-hydroxyphenyl)-L-alanine and **2** [9]. **1** has also been identified in *Combretum zeyheri* (Combretaceae). In a preliminary communication L-configuration is stated but without rotation values [10]. In the same species was found **2** and 3-(3-aminomethylphenyl)-L-alanine [10, 11]. The co-occurrence of **1** and **2** in these three plant families indicates a metabolic inter-relationship. **2** is known to be derived from shikimic acid in *Reseda* species [8]. **2** has been found in many plant families including Iridaceae, Leguminosae and Combretaceae as mentioned above but also Resedaceae [8], Cruciferae [12], and Cucurbitaceae [13].

EXPERIMENTAL

Instrumentation. Optical rotations were determined with a Perkin–Elmer 141 photoelectric polarimeter (1 dm tubes). CD-curves were recorded with a Roussel–Jordan CD 185 Dichrographe in N HCl. PMR spectra were determined in D_2O with Na 2,2,3,3-tetradeuterio-3-(trimethylsilyl)propionate as int stand.