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

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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.

Plant material. Leaves of *I. sanguinea* Donn were obtained from the Botanical gardens of the Universities of Aarhus and Copenhagen in the summer of 1977. The seeds of *I sibirica* L. were obtained from the Botanical Garden of the University of Copenhagen, harvested in the autumn of 1971.

Isolation of **1** *from* I. sanguinea *leaves.* Fr. leaves (500 g) were homogenized in CCl_4 and subsequently extracted twice with CCl_4 and twice with 70% MeOH. The combined MeOH–H_2O extracts were evapd to dryness (16 g), dissolved in H_2O and in three portions applied to a strongly acid ion-exchange resin (Amberlite IR 120, H^+, 3 × 90 cm). After washing with H_2O, the amino acids were eluted with N NH_3. The combined ninhydrin reacting fractions were concd to dryness (2.9 g), dissolved in H_2O and applied to a strongly basic ion-exchange resin (Dowex 1, 200–400 mesh, AcO^-, 3 × 60 cm). The effluent from this column, containing neutral and basic amino acids was concd to dryness (2.1 g), the residue dissolved in H_2O and applied to a column of carbon deactivated with stearic acid [14] (3 × 13 cm). The non-aromatic amino acids were eluted with H_2O to give 1.8 g, and the aromatic amino acids were eluted with PhOH–AcOH–H_2O (2:5:23) to give 0.6 g. The fraction was applied to a strongly acid ion-exchange resin (Dowex 50 W × 8, 200–400 mesh, H^+, 1 × 35 cm) and the aromatic amino acids were eluted with N Py. The fractions containing **1** were combined and evapd to dryness to give 300 mg paper-chromatographically pure material. 160 mg of this material was applied to a Sephadex G-10 column (3 × 90 cm) and eluted with H_2O. The fractions containing **1** were evapd to dryness, and after addition of EtOH to the solid residue, colourless crystals were collected by filtration (50 mg) (in addition 60 mg of weakly coloured material was obtained). Earlier attempts at recrystallization of the amino acid have been unsuccessful [9], but we achieved this by soln of 32 mg in H_2O (0.4 ml), filtration, addition of EtOH (10 ml) and cooling for three weaks to give 17 mg pure material. $[\alpha]_D^{22}$ −25.5° (*c* 0.8, H_2O), $[\alpha]_D^{20}$ −5.5° (*c* 0.6; N HCl). UV: λ_{max} 212 and 260 nm. PMR: δ 3.2 ppm (CH_2 in alanine side chain), 4.02 (CH in alanine side chain), 4.65 (CH_2OH), 7.4 (aromatic protons). MS (solid inlet, probe 220°, 70 eV): 195 (M^+), 177, 150, 132, 121, 104, 92, 74. The IR spectrum of the sample in KBr showed minor differences from that of the synthetic racemate. The fraction of acidic amino acids eluted from the Dowex 1 column with N HOAc (500 mg) contained **2** and **3** as demonstrated by PC.

Isolation of **1** *from* I. sibirica *seeds.* The isolation was performed as above from 17 g of seeds. The total amino acid fraction (240 mg) yielded 34 mg of **1**. $[\alpha]_D^{22}$ −20.9 (*c* 1.2; H_2O), $[\alpha]_D^{22}$ −5.8° (*c* 1.0; N HCl). The isolate showed a PMR spectrum undistinguishable from that described above both with regard to chemical shifts, coupling patterns and intensities.

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BIOSYNTHESIS OF γ-AMINOBUTYRIC ACID FROM SPERMINE IN MAIZE SEEDLINGS

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Key Word Index—*Zea mays*; Gramineae, maize; biosynthesis; γ-aminobutyric acid; spermine.

Abstract—The administration of labelled spermine [tetramethylene-1,4-^{14}C] to *Zea mays* shoots resulted in the formation of radioactive γ-aminobutyric acid (GABA). A chemical degradation of radioactive GABA suggested that its radioactivity was located on C-1 and C-4, indicating that GABA is a product of spermine metabolism in maize seedlings.

INTRODUCTION

The oxidative degradation of spermine and spermidine by the plant polyamine oxidase was first described by Smith [1]. These studies showed that 1,3-diaminopropane is one product of this oxidative degradation. More recently we have shown the formation of β-alanine from 1,3-diaminopropane in maize seedlings [2]. Δ^1-