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-

Pyrroline, the other product of spermidine oxidation by polyamine oxidase [3, 4], was oxidized easily to γ-aminobutyric acid (GABA) by a plant peroxidase system (unpublished). In this paper we report the formation of GABA from spermine in maize seedlings.

RESULTS AND DISCUSSION

The results of the biosynthetic experiment are presented in Table 1. Besides unconverted spermine, the major radioactivity was found in 1-(3-aminopropyl)pyrroline and GABA. However, some label was also found in an unidentified substance. This shows that spermine is metabolized to GABA in maize seedlings. The radioactive GABA (6300 dpm) was isolated by TLC (Si gel G; R_f 0.38 solvent 1) with 80% EtOH. It was converted to *p*-toluenesulfonyl derivative with carrier GABA. After recrystallization, the pure white crystalline product was shown to have a constant activity (5600 dpm). The incorporation percentages in Table 1 suggested that spermine can be converted into GABA via 1-(3-aminopropyl)pyrroline. The radioactive GABA was degraded chemically [5]. The activities of these degradation products are recorded in Table 2. These results indicate that essentially all activity in GABA was located on C-1 and C-4. This result is consistent with the formation of GABA from spermine by cleavage of the C—N bond of 1-(3-aminopropyl)pyrroline. Spermine and spermidine are therefore mainly metabolized into GABA and β-alanine in the maize seedlings.

Table 1. Distribution of radioactivity(^{14}C) in γ-aminobutyric acid and 1-(3-aminopropyl)pyrroline in excised maize shoots

Fraction	Days after feeding					
	1		2		3	
	dpm	(%)	dpm	(%)	dpm	(%)
Spermine	11600	(58)	10300	(46)	9830	(42)
1-(3-Aminopropyl)-pyrroline	3330	(17)	5120	(23)	5910	(25)
γ-Aminobutyric acid	523	(3)	1070	(5)	1140	(5)
Unidentified substance	511	(3)	863	(4)	953	(4)
Total activity	20000	(100)	22200	(100)	23600	(100)

Extracts (20 mg fr. wt) were developed by PC.

EXPERIMENTAL

General methods. A liquid scintillation counter was used for assay of the radioactive compounds using PPO–dimethyl-POPOP–toluene scintillator. Dioxane scintillator was used for assay of 1,2-diaminoethane and 1,3-diaminopropane.

Feeding experiment. This was carried out as in ref. [2]. The shoot tips were grown 2 weeks under continuous light.

Extraction and fractionation. Radioactive compounds from green shoot tips were determined with the EtOH-soluble fraction obtained by extraction with 99% EtOH as described previously [2]. The R_fs in solvent 1 [2] were spermine, 0.05: 1-(3-aminopropyl)-pyrroline, 0.11: GABA, 0.46.

Table 2. Radioactivity of γ-aminobutyric acid and its degradation products

		Radioactivity (dpm)
First degradation	γ-aminobutyric acid	5000
	$BaCO_3$	1630
	1,3-diaminopropane	1180
Second degradation	γ-aminobutyric acid	5000
	Succinic acid	2540
	$BaCO_3$	1240
	1,2-diaminoethane	141

0.2 g of unradioactive GABA was used as carrier substance in each case.

Degradation of the carbon chain of radioactive GABA. Distribution of isotopic carbon in GABA was determined by the Schmidt reaction [5]. GABA was first subjected to the Schmidt reaction to form CO_2 and 1,3-diaminopropane. Secondly, GABA was oxidized to succinic acid with $KMnO_4$. After separating the excess $KMnO_4$ by Na_2SO_3 treatment, succinic acid (50% yield) was isolated from unreacted GABA using an Amberlite IR-120 (H^+) column. After the radioactivity was counted, the succinic acid was degraded to CO_2 and 1,2-diaminoethane (total 55% yield) by the Schmidt reaction. The produced CO_2 was converted to $BaCO_3$ using $Ba(OH)_2$ soln. To recover 1,2-diaminoethane and 1,3-diaminopropane which were formed in the reaction mixture, 0.2 N HCl was used as the trapping agent.

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