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CYCLOPROPYLAMINO ACIDS OF THE GENUS *ACER*: DISTRIBUTION AND BIOSYNTHESIS

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Key Word Index—*Acer* spp.; Aceraceae; cyclopropylamino acids; chemotaxonomy; hypoglycin-*A* biosynthesis.

Abstract—A survey has been made of amino acids and γ-glutamyl peptides present in seeds of many *Acer* species. Particular emphasis was placed upon the occurrence of cyclopropylamino compounds, and their pattern of distribution is examined against a recent revised classification of the genus. Labelled precursor feeding experiments have been used to provide preliminary information about the pathways involved in hypoglycin-*A* biosynthesis in *A. pseudoplatanus* (sycamore) fruits.

INTRODUCTION

WE RECENTLY reported the isolation of β-(methylenecyclopropyl)alanine (hypoglycin-*A*) and α-(methylenecyclopropyl)glycine and their derived γ-glutamyl peptides from sycamore fruits (*Acer pseudoplatanus*).[1] The two cyclopropylamino acids and hypoglycin-*B* (the γ-glutamyl peptide of hypoglycin-*A*) had been described earlier[2–4] as constituents of several members of the related families, Sapindaceae and Hippocastanaceae, but γ-glutamyl-α-(methylenecyclopropyl)glycine is a new compound which was also isolated from seeds of *Billia hippocastanum*.[1] The present paper describes the extension of our study of these constituents to other *Acer* species; the results of this survey have been examined against a new classification of the genus.[5] We also report the results of preliminary experiments on the biosynthetic precursors of hypoglycin-*A* in developing sycamore fruits, and evaluate these observations in relation to biogenesis in developing fruits of *Aesculus californica*,[6,7] which synthesize 2-amino-4-methylhex-4-enoic acid (AMHA, another branched-chain C_7 amino acid).

RESULTS AND DISCUSSION

Distribution Studies

The amino acid composition of aqueous ethanol extracts of fruits of various *Acer* species were examined principally by 2-D PC. The presence of the γ-glutamyl peptides of α-(methylenecyclopropyl)glycine and hypoglycin-*A* were confirmed by paper electrophoresis at pH 3·4,[1] and that of hypoglycin-*A* itself by catalytic hydrogenation followed by identification of the characteristics mixture of homoleucine reduction products.[4]

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TABLE 1. THE OCCURRENCE OF CYCLOPROPYLAMINO ACIDS WITHIN MEMBERS OF THE GENUS *Acer*

Species arranged under subgenus, section and series after Murray[5]	α-(Methylene-cyclopropyl) glycine	γ-Glutamyl-α-(methylene-cyclopropyl) glycine	Hypoglycin-*A*	Hypoglycin-*B*
Subgenus Acer				
Section Acer				
Series Acer				
*Acer pseudoplatanus**	W	S	MS	S[a]
A. pseudoplatanus Cv. *rubrum**	T	S[a]	M	S[a]
A. pseudoplatanus Cv. *variegatum*†	W	MS	M	S
Section Distyla				
Series Distyla				
*A. distylum**	M	WM	WM	WM
Section Ginnala				
Series Tatarica				
A. ginnala†	O	O	O	O
A. tataricum§	O	O	O	O
Section Glabra				
Series Glabra				
A. glabra subsp. *douglasii*†	O	O	O	O
Section Macrantha				
Series Tegmentosa				
A. pensylvanicum†	O	O	O	O
A. hersii (= *A. tegmentosum* subsp. *grosseri*)‡	O	O	O	O
A. rufinerve‡	O	O	O	O
Series Crataegifolia				
*A. davidii**	O	O	O	O
Section Macrophylla				
Series Macrophylla				
A. macrophyllum†	T	W	WM	WM
Section Palmata				
Series Palmata				
A. palmatum‡	O	O	M	S[a]
A. palmatum subsp. *matsumurae*‖	O	O	W	S[a]
A. palmatum Cv. *atropurpureum*‡	O	O	WM	S
*A. sieboldianum**	W	O	WM	S
*A. japonicum**	O	O	WM	S
Section Platanoidea				
Series Campestria				
A. campestre‡	O	O	O	O
Series Platanoidea				
A. platanoides‡	O	O	O	O
A. platanoides Cv. *schwedleri*§	O	O	O	O
*A. cappadocicum**	O	O	O	O
Section Spicata				
Series Spicata				
A. spicatum†	T	MS	M	S
Section Trifoliata				
Series Grisea				
A. griseum‡	O	O	O	O
Subgenus Eriocarpa				
Section Eriocarpa				
Series Eriocarpa				
A. saccharinum Cv. *laciniatum weiri*§	W	WM	WM	M
Section Rubra				
Series Rubra				
A. rubrum†	O	O	O	O
Subgenus Negundo				

TABLE 1—*continued*

Species arranged under subgenus, section and series after Murray[5]	α-(Methylene-cyclopropyl) glycine	γ-Glutamyl-α-(methylene-cyclopropyl) glycine	Hypoglycin-*A*	Hypoglycin-*B*
Section Negundo				
Series Negundo				
A. negundo†	WM	T	MS	M
A. negundo Cv. *auratum*§	M	M	MS	S
A. negundo Cv. *variegatum*‡	WM	M	WM	WM
Subgenus Saccharodendron				
Section Saccharodendron				
Series Saccharodendron				
A. saccharum†	W	W	MS	S
A. saccharum subsp. *nigrum*†	W	W	M	S

Relative concentrations of individual compounds on chromatograms are denoted by: S—strong; M—moderate; W—weak; T—trace; O—not detected: [a] indicates that compound formed predominant spot on particular chromatogram.

Seed samples were obtained as follows:

* Westonbirt Arboretum via Royal Botanic Gardens, Kew.

† Petawawa Forest Experiment Station, Chalk River, Ontario, Canada.

‡ Thompson & Morgan Ltd., Ipswich.

§ Forest Experiment Station, Timisoara, Pădurea Verde, Romania.

‖ Dr. I. Murakoshi, Chiba, Japan.

The results of the survey for cyclopropyl derivatives in the different species are shown in Table 1. The species are arranged following Murray's classification,[5] which divides the *Acer* genus into seven subgenera, and lower sections and series. We have examined species from four of these subgenera. The subgenus Acer contains most species and was split into 16 sections by Murray: species assigned to 10 of these sections have been analyzed. Species assigned to each of the sections of the subgenera Eriocarpa and Saccharodendron were available, while one of two sections of the subgenus Negundo was represented. The ability to synthesize the cyclopropylamino acids is clearly restricted to the members of certain sections of the genus: furthermore, when several species from an individual series were available for analysis, all showed a common pattern of amino acid accumulation. In species where synthesis takes place, all four cyclopropyl compounds usually co-existed, but the γ-glutamyl peptides normally accumulated in higher concentrations than the corresponding free amino acids. Species assigned to the section Palmata formed an exception since they contained large amounts of hypoglycins-*A* and -*B* but usually lacked detectable amounts of α-(methylenecyclopropyl)glycine and its peptide.

The survey revealed more than 30 other ninhydrin-positive compounds as minor, unidentified constituents of the *Acer* spp. extracts. The distribution of most of these compounds between species did not conform to any regular pattern when viewed against Murray's classification. However, a compound giving a bright yellow chromophore with ninhydrin, and occurring near the position of phenylalanine on standard two-dimensional paper chromatograms, formed a significant component of the extract from a single species, *A. distylum*. This species was raised by Murray[1] to the level of a separate monotypic series forming the section Distyla of the subgenus Acer. He observed that it differed morphologically from the section Macrantha in having buds with few scales (not valvate), leaves cor-

date-crenate (not ovate-lobate-serrate) and samaras ascendent (not subhorizontal)'. Monotani[8] had reported previously that *A. distylum* exhibited certain unique features of seed protein composition. There is then agreement concerning the distinctness of this species, with supporting evidence coming from morphological studies, and from both macromolecular and micromolecular chemical approaches. We hope to acquire a quantity of *A. distylum* material sufficient to permit the isolation and characterization of the 'unidentified' compound.

TABLE 2. THE INCORPORATION OF ^{14}C-LABELLED COMPOUNDS INTO HYPOGLYCIN-*A* BY DEVELOPING FRUITS OF *Acer pseudoplatanus*

Compound supplied	% ^{14}C Incorporation into hypoglycin-*A*	Compound supplied	% ^{14}C Incorporation into hypoglycin-*A*
L-[U-^{14}C]Isoleucine (20 μCi)	0·020	D-[U-^{14}C]Glucose [100 μCi]	0·007
L-[U-^{14}C]Leucine (20 μCi)	0	[U-^{14}C]Acetate [100 μCi]	0·051
L-[methyl-^{14}C]Methionine (20 μCi)	0·118		

Precursor Labelling Experiments

The ability of several ^{14}C-labelled compounds to act as precursors of carbon atoms of the hypoglycin-*A* skeleton were examined during the phase of rapid growth of sycamore seeds, i.e. in mid-August. The labelled compounds were infiltrated, via cotton wicks,[6] into fruit stalks carrying 16–20 healthy developing seeds. Uptake of labelled solution normally was complete in 24 hr but metabolism of the ^{14}C-compounds was allowed to continue for a further 5 days. The fruits then were collected, extracted and analyzed for ^{14}C-hypoglycin-*A*. Since hypoglycin-*A* is not resolved from leucine/isoleucine by either PC or Technicon amino acid autoanalysis each extract was hydrogenated to yield a mixture of reduction products which could be readily resolved from hypoglycin-*A* and the leucines on PCs developed with *tert*-amyl alcohol–acetic acid–water mixture. The extent of ^{14}C-labelling was determined using a Packard radiochromatogram scanner.

Table 2 presents the results showing the percentage incorporation of ^{14}C into hypoglycin-A from five labelled compounds. These percentage conversions are low when compared with similar values determined for AMHA biosynthesis in *Aesculus californica*. The highest incorporation was from [methyl-^{14}C]methionine, followed by [U-^{14}C]acetate. These observations might suggest a biogenetic pathway in which acetate undergoes initial condensation to yield a linear C_6 skeleton, and that the cyclopropyl ring originated by C_1 addition of the methyl group of methionine at a position of 4,5-unsaturation. Isoleucine carbon was poorly assimilated into hypoglycin-*A* in contrast to AMHA where [U-^{14}C]isoleucine was incorporated about 10 times more efficiency than any other ^{14}C-labelled precursor tested. This does not support the idea that the two C_7 amino acids may share common early biosynthetic steps. Our results with sycamore closely resembled data obtained by Dr. R. Suhadolnik (Philadelphia, personal communication) in a study of hypoglycin-*A* biosynthesis in developing fruits of *Blighia sapida*, where acetate and the methyl group from methionine again

[8] MONOTANI, Y. (1962) *Mem. Coll. Sci. Univ. Kyoto* **29B**, 81.

represent the most effective precursors of the C skeleton of hypoglycin-*A*. Clearly, further studies using additional precursors labelled on specific C atoms are necessary before detailed biogenetic hypothesis can be formulated.

EXPERIMENTAL

Amino acid distribution. Samples of *Acer* seed were finely ground and extracted with 70% (v/v) EtOH. Amino acid fractions were separated using small cation-exchange resin columns, and the individual components resolved on 2-D PCs (phenol–NH_3 followed by BuOH-HOAc–H_2O, 4:1:5).[9] Electrophoretic techniques to confirm the identity of the γ-glutamyl peptides were based on those described earlier.[1]

*Radioisotopic study of hypoglycin-*A *biosynthesis.* Labelled compounds were purchased from the Radiochemical Centre, Amersham, and adjusted to a specific activity of 10 μCi/μmol before use. Streptomycin (20 μg/ml) was included in each labelled solution before infiltrating into the fruit stalks. Procedures for the extraction, separation and assay of labelled amino acids were based on those used in previous investigation of AMHA biosynthesis.[6] Radioactivity associated with hypoglycin-*A* was present in β-(methylcyclopropyl)-alanine and homoleucines after catalytic hydrogenation of amino acid fractions.

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