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BIOSYNTHESIS OF 3-METHOXYCARBONYLPROPYL-GLUCOSINOLATE IN AN *ERYSIMUM* SPECIES*

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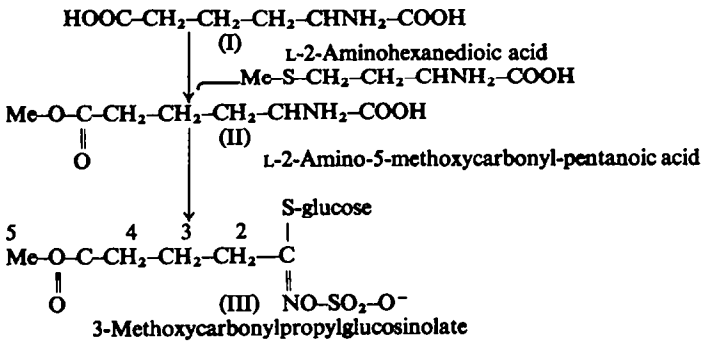
Key Word Index—*Erysimum rupestre*; Cruciferae; 3-methoxycarbonylpropylglucosinolate; DL-2-amino-hexanedioic acid; DL-2-amino-5-methoxycarbonyl-pentanoic acid; biosynthesis.

Abstract—Incorporation of DL-2-aminohexanedioic acid, DL-2-amino-5-methoxycarbonyl-pentanoic acid and DL-methionine into 3-methoxycarbonylpropylglucosinolate have been demonstrated using an *Erysimum* species. The data support the following sequence of biosynthetic reactions: 2-aminohexanedioic acid is methylated by methionine; the resulting 2-amino-5-methoxycarbonyl-pentanoic acid is then converted into the glucosinolate. 2-Amino-5-methoxycarbonyl-pentanoic acid has been tentatively identified as a natural product in the plant.

INTRODUCTION

3-METHOXYCARBONYLPROPYLGLUCOSINOLATE (III, see Scheme 1) was isolated from an *Erysimum* species in 1957 by Kjaer and Gmelin.[1] Its side chain is unique among the glucosinolates, in that it is the only one, on the basis of structural analogy, that would likely be derived from a dicarboxylic amino acid. Ettlinger and Kjaer[2] have noted that the side chain of this glucosinolate corresponds to 2-aminohexanedioic acid (I), which is widely distributed in higher plants[3–5] including the family of Cruciferae.



SCHEME 1. PROPOSED BIOSYNTHESIS OF 3-METHOXYCARBONYLPROPYLGLUCOSINOLATE.

This publication reports the incorporation of the following ^{14}C-labeled compounds: acetate, aspartic acid, glutamic acid, methionine, 2-aminohexanedioic acid (I) and 2-

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[1] A. KJAER and R. GMELIN, *Acta Chem. Scand.* **11**, 577 (1957).
[2] M. G. ETTLINGER and A. KJAER, in *Recent Advances in Phytochemistry* (edited by T. MABRY *et al.*), Vol. 1, p. 59, Appleton–Century–Crofts, New York (1968).
[3] S. I. HATANAKA and A. I. VIRTANEN, *Acta Chem. Scand.* **16**, 514 (1962).
[4] P. O. LARSEN, *Acta Chem. Scand.* **19**, 1071 (1965).
[5] L. FOWDEN, *Progress in Phytochemistry*, Vol. 2, p. 203, Interscience Publishers, London (1971).

amino-5-methoxycarbonyl-pentanoic acid (II) into 3-methoxycarbonylpropylglucosinolate (III) by the plant. The results are presented in Table 1. They support the proposed biosynthetic scheme depicted in Scheme 1.

TABLE 1. COMPARISON OF ^{14}C-LABELED COMPOUNDS AS PRECURSORS OF 3-METHOXYCARBONYLPROPYLGLUCOSINOLATE

	Compound administered			Compound isolated			
	Total nCi	Sp. act. nCi/mmol	Total mmol	Total nCi	Sp. act. nCi/mmol	Dilution* value	% Incorporation†
Acetate-2-^{14}C	8200	820 000	0·330	1·7	5·0	165 000	0·02
DL-Aspartic acid-G-^{14}C	1500	150 000	0·157	7·5	48·0	3130	0·50
DL-Glutamic acid-G-^{14}C	2200	220 000	0·113	11·5	102·0	2158	0·52
DL-Methionine-$^{14}CH_3$	2200	220 000	0·127	174·0	1370·0	160	7·90
DL-2-Aminohexanedioic acid-2-^{14}C	2200	220 000	0·145	388·0	2680·0	82	17·60
DL-2-Aminohexanedioic acid-6-^{14}C	2200	220 000	0·120	366·0	3045·0	72	16·60
DL-2-Amino-5-methoxycarbonyl-pentanoic acid-2-^{14}C	2200	220 000	0·165	656·0	3970·0	55	28·00

* Dilution value = sp. act. of compound fed (nCi/mmol)/sp. act. of compound isolated (nCi/mmol).
† % ^{14}C-incorporated = total nCi in compound × 100/nCi administered.

RESULTS AND DISCUSSION

The plants used in this investigation were grown from seed which was obtained from the Museum National d'Histoire Naturelle, 43 rue de Buffan, Paris, France. It was labeled *Erysimum rupestre* DC. Gmelin and Kjaer[6] later indicated that the previously reported sources of 3-methoxycarbonylpropylglucosinolate was *E. odoratum* Ehrh. Originally they were misled by an erroneous identification of *E. odoratum* as *E. rupestre*.[1] This leaves the botanical diagnosis of the plant used in this study in question. An investigation is currently underway to positively identify this plant. The findings will be reported later.

Potential precursors of 3-methoxycarbonylpropylglucosinolate (III) are: acetate, aspartic acid, glutamic acid, 2-aminohexanedioic acid (I), the methyl group of methionine and 2-amino-5-methoxycarbonyl-pentanoic acid (II). Each of these compounds was administered to a different lot of plants. After a 24-hr metabolic period the isothiocyanate of 3-methoxycarbonylpropylglucosinolate (III) was quantitatively determined in an aliquot of the plant extract by the GLC method of Youngs and Wetter.[7] The specific radioactivity was determined as described in the Experimental; using these two values, the percent incoporation was calculated.

From the results presented in Table 1 it is apparent that methionine-^{14}Me, 2-aminohexanedioic acid-2-^{14}C (I), 2-aminohexanedioic acid-6-^{14}C (I), and 2-amino-5-methoxycarbonyl-pentanoic acid-2-^{14}C (II) are efficient precursors of 3-methoxycarbonylpropylglucosinolate (III). The increasing percentage incorporation and the decreasing dilution values support the biosynthetic scheme proposed in Scheme 1. 2-Aminohexanedioic acid-2-^{14}C (I) and 2-aminohexanedioic acid-6-^{14}C (I) were converted to the glucoside with equal efficiency, suggesting that the integrity of the amino acid carbon skeleton was retained. The role of methionine in biological methylation is well known; it is without doubt acting as a methyl group donor in this system. Of the compounds fed, 2-amino-5-methoxycarbonyl-pentanoic acid (II) was the one most efficiently converted to the glucoside, a result which suggests that the 2-aminohexanedioic acid (I) is methylated and then utilized.

[6] R. GMELIN and A. KJAER, *Acta Chem. Scand.* **23**, 2548 (1969).
[7] C. G. YOUNGS and L. R. WETTER, *J. Am. Oil Chem. Soc.* **44**, 551 (1967).

The efficiency with which glutamic acid-G-^{14}C, aspartic acid-G-^{14}C and acetate-2-^{14}C were converted to 3-methoxycarbonylpropylglucosinolate (III) suggests that both glutamic acid and aspartic acid are likely precursors, but that acetate is probably not involved. This is in contrast to the amino acid precursors of other glucosinolates which are formed from their lower homologs by a series of reactions that includes the condensation of a keto acid with acetate and subsequently form an amino acid with one methylene carbon more than the original.[8,9]

2-Amino-5-methoxycarbonyl-pentanoic acid (II) has been tentatively identified among the free amino acids extracted from untreated plant material. It eluted from the column of a Beckman Amino Acid Analyzer, model 120c, with the same retention time as an authentic sample.

EXPERIMENTAL

Cultivation of plants and administration of labeled compounds. The experiments were performed on mature plants that had been growing in a greenhouse for 3–4 months. The radioactive compounds were administered through the cut ends of the stocks as described previously.[10] Approximately 10 μmol of radioactive compound was administered for every 10 g of fresh plant material. The metabolic period was 24 hr under 100 lx of continuous light.

Radioactive compounds. Acetate 2-^{14}C, aspartic acid G-^{14}C, glutamic acid G-^{14}C, methionine-^{14}Me and 2-aminohexanedioic acid-6-^{14}C were obtained from commercial sources. 2-Aminohexanedioic acid 2-^{14}C was synthesized by the reaction of diethylacetomidomalonate-2-^{14}C, 4-bromobutyronitrile and sodium in EtOH. The product diethylacetomidobutyronitrilemalonate was hydrolyzed by refluxing for 18 hr in 6 N HCl. The 2-aminohexanedioic acid was recovered and purified by ion exchange chromatography.[11] 2-Amino-5-methoxycarbonyl-pentanoic acid-2-^{14}C was prepared by the method of Augustin.[12] To a well-stirred suspension of 2-aminohexanedioic acid-2-^{14}C (161 mg) in 3 ml MeOH at −15° was added 0·0715 ml $SOCl_2$. After the addition, the temp. was allowed to rise slowly to 21° where it was retained for a further 25 hr. After removing the solvent *in vacuo* the amino acids were absorbed on a column of ion exchange resin (Amberlite 1R 120 H^+) and the column was washed free of inorganic acid with H_2O. Then the amino acids were eluted from the resin with 2 N NH_4OH. The NH_4OH was evaporated and the 2-amino-5-methoxycarbonyl-pentanoic acid (II) was separated from the residual 2-aminohexanedioic acid (I) by eluting them from a column (2 × 60 cm) of Dowex 1 X8 acetate resin with 0·5 N HOAc. The 2-amino-5-methoxycarbonyl-pentanoic acid (II) eluted first yield 159 mg, 91%.

Isolation and identification. 3-Methoxycarbonylpropylglucosinolate (III) was isolated and purified as described previously.[13] The glucoside was obtained as a glass hard syrup; repeated attempts to crystallize it failed. Using the TLC method of Matsuo[14] only one compound was shown in each of four solvents. The NMR spectrum at 100 MHz in D_2O, using tetramethylsilane as an external reference, showed signals at σ 2·52 (*q*, *J* 7·5 Hz, 3-C protons); 3·04 (*t*, *J* 7·5 Hz, C-2 protons); 3·28 (*t*, *J* 7·5 Hz, C-4 protons) and 4·28 (*s*, C-5 protons), (see Scheme 1 for numbering system). Appropriate signals for the glucose protons were also present. The assignments were supported by integration and no proton signals from contaminating compounds were detected. An IR spectrum (in KBr) showed a strong band at 1725 cm^{-1} which is characteristic of an ester group. A NMR spectrum of 3-methoxycarbonylpropyl isothiocyanate recovered from an enzyme hydrolysis of the glucosinolate and of a synthetic sample prepared by the method of Kjaer and Gmelin[1] were identical. Their retention times on two GLC columns were also the same. In the biosynthetic study the total chlorophyll free extract was used. A 5% aliquot of the extract was treated with thioglucosidase (E.C. 3.2.3.1, thioglucoside glucohydrolase), and the isothiocyanate released was quantitatively assayed by the GLC.[7] A Hewlewlett–Packard gas chromatograph, model 5754, was used. It carried a 150 × 0·32 cm o.d. stainless-steel column packed with 20% FFAP on acid washed, DMCS-treated, chromosorb W, 60–70 mesh; helium flow 30 ml/min; hydrogen 20 ml/min; injector and detector were 250°. At an oven temp. of 200°, 3-methoxycarbonylpropyl isothiocyanate had a retention time of 0·54 relative to 2-phenylethyl isothiocyanate. The remaining 95% of the extract was used to prepare purified isothiocyanate for counting;

[8] M. D. Chisholm and L. R. Wetter, *Can. J. Biochem.* **42**, 1033 (1964).
[9] E. W. Underhill, *Can. J. Biochem.* **46**, 401 (1968).
[10] E. W. Underhill, M. D. Chisholm and L. R. Wetter, *Can. J. Biochem. Physiol.* **40**, 1505 (1962).
[11] M. D. Chisholm and L. R. Wetter, *Can. J. Biochem.* **44**, 1625 (1966).
[12] M. Augustin, *Chem. Ber.* **99**, 1040 (1966).
[13] M. D. Chisholm, *Phytochem.* **11**, 197 (1972).
[14] M. Matsuo, *J. Chromatog.* **49**, 323 (1970).

a 10:1 stream splitter was added to the GLC apparatus and the isothiocyanate was collected. The amount collected was assayed, its radioactivity was determined and its specific activity calculated.

Isotope analyses. Radioactive samples were assayed as described earlier.[13]

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