acid **4** (in which the asymmetric centre is removed even farther from the carboxyl), the difference between the rates of hydrolysis of the 2 enantiomers is not great enough to allow an optical purity useful for preparative purposes to be obtained. A comparison of the specific rotations found with the respective maximum values reported in the literature, shows, in fact, that the optical purity is about 40% for the δ-amino-acid **3** and 35% for the ε-amino-acid **4**. Passing from the γ-amino derivative to the subsequent ones seems then to be critical for the stereoselectivity of the hydrolysis in the conditions adopted.

Examination of the data in the table clearly shows that, for all the substrates examined, BPA preferentially hydrolyzes the S-enantiomers. This stereoselectivity is not lost even in the case of the hydrolysis of the N-PA-derivative of α-methyl-β-alanine **7**, in which the acylamino group is not directly bonded to the chiral centre. A significant consequence of the above observation concerns the possibility of evaluating the opposite assignments[9-11], made via chemical correlations, of the absolute configuration to γ-aminovaleric acid **2**, a compound of current chemical and biochemical interest. The hydrolysis data of the table are in accordance with the configurational assigment R(+) made previously by us[9]; this assignment is reported in the table. The statement made by other authors[10,11] according to which in the series 'alanine, β-aminobutyric acid, γ-aminovaleric acid', the enantiomers possessing the same sign of optical rotation, have the same configuration, finds no support, even on biochemical grounds.

As far as the rates of hydrolysis are concerned, 2 preliminary observations can be made regarding the influence of the distance of the amido group from the carboxylic group and the substitutions at the asymmetric carbon atom. The hydrolysis rates seem to be more influenced by the hindrance of the substituents at the asymmetric carbon atom than by the progressive removal of the same carbon atom from the carboxylic group. This effect becomes evident if the incubation times for compounds **1**–**4** are compared with those for compounds **1** and **6** and for compounds **1** and **5** (table).

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Methionine metabolism in apple tissue

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Summary. A metabolic intermediate isolated from apple tissue fed either methionine or 5′-methylthioadenosine has been tentatively identified as a methionine-pyridoxal Schiff base. The formation of this compound is discussed in relation to ethylene biosynthesis.

Methionine has been established as a precursor of ethylene in a number of fruit and vegetative tissues, but it is not the immediate precursor[2]. In apple tissue recent evidence suggests that methionine is converted into S-adenosylmethionine prior to its conversion to ethylene[3-5], that pyridoxal phosphate is involved as coenzyme[4-6], and that 5′-methylthioadenosine is a fragment nucleoside product[4,5,7]. The formation of 5′-methylthioadenosine is consistent with the observation that no volatile sulfur compounds are recovered during the conversion of methionine to ethylene[8,9].

Although methionine is an excellent precursor of ethylene in fruit tissue, there is a lag period of about 40 min before a steady rate of ethylene production from methionine is observed[10]. It has beeen noticed that apple tissue produced little ethylene in a nitrogen atmosphere, but that ethylene production was greatly stimulated upon transfer to air[11]. These results suggest that an intermediate(s) is (are) involved in the conversion of methionine to ethylene which accumulates in the presence of nitrogen and is degraded rapidly in air. Although there has been speculation as to the possible intermediate(s) in ethylene synthesis from methionine[2], there have been no reports concerning isolation and identification of the intermediate(s). This paper describes the formation of methionine-pyridoxal Schiff base from either methionine-[Me-^{14}C] or 5′-methylthioadenosine-[Me-^{14}C] fed to apple tissue.

Materials and methods. Apples (cv. Golden Delicious) were purchased from a local market. S-Adenosyl-L-methionine-[Me-^{14}C] was purchased from New England Nuclear. 5′-Methylthioadenosine-[Me-^{14}C] was prepared by hydrolysis of S-adenosyl-L-methionine-[Me-^{14}C] for 20 min at 100 °C in dilute HOAc, pH 4.0[12]. Verification that only labelled 5′-methylthioadenosine was present in the hydrolyzate was done by paper co-chromatography and co-electrophoresis with authentic sample as described previously[5]. Methionine-[Me-^{14}C] was a product of Amersham/Searle. Apple plugs (1 cm in diameter and 2 cm in length) were cut from the fruit with a cork borer and razor blade. The

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Table 1. Distribution of ^{14}C label in metabolites isolated from apple tissue fed 5′-methylthioadenosine-[Me-^{14}C]

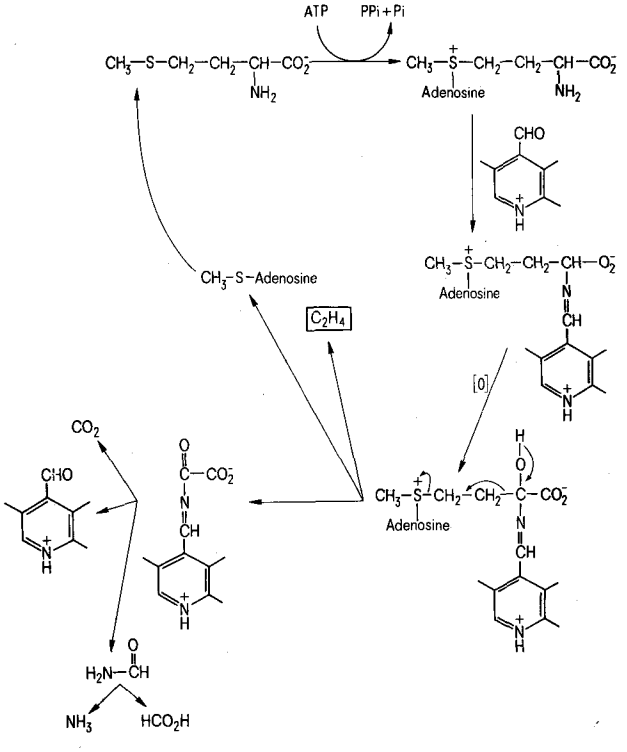
Compound	R_f	Percent of total ^{14}C recovered Exp. 1	Exp. 2
Unknown A	0.04	9.8	10.9
Methionine	0.42	37.4	38.0
5′-methylthioadenosine	0.66	40.1	36.5
Unknown B	0.87	11.6	14.6

Apple plugs (1 × 2 cm) were fed 100 µl 2% KCl solution containing 0.6 µCi 5′-methylthioadenosine-[Me-^{14}C] and incubated 3 h in air at 20 °C.

Table 2. Distribution of ^{14}C label in metabolites isolated from apple tissue fed methionine-[Me-^{14}C]

Compound	Amount of ^{14}C relative to air Air	N_2	Air+0.1 mM canaline	Air+0.1 mM DNP
Unknown A	1.00	1.47	0.65	1.41
5′-methylthioadenosine	1.00	0.32	0.61	0.38

Apple plugs (1 × 2 cm) were fed 100 µl 2% KCl solution containing 2.8 µCi methionine-[Me-^{14}C] or methionine-[Me-^{14}C] plus inhibitors of ethylene synthesis. The tissue was incubated 5 h in air or N_2 at 20 °C.



Scheme proposed by Yang and Baur[6] for biosynthesis of ethylene from methionine mediated by pyridoxal phosphate, and subsequently modified by Murr and Yang[4] to indicate a role for ATP in the formation of S-adenosylmethionine.

radioactive substrates in 2% KCl solution were introduced into the plugs by a vacuum infiltration technique[13]. After incubation, the tissues were extracted with 10 ml cold 80% ethanol containing 0.2% mercaptoethanol and 0.1 mmoles sodium borohydride, which reduces any Schiff base that may have been formed into the stable secondary amine[14]. The extract was incubated overnight at 40 °C with mercaptoethanol to convert any methionine sulfoxide to methione, and then the volume was reduced to about 2 ml in vacuo.

An aliquot of the extract was streaked on Whatman 1 paper and developed overnight in BuOH-HOAc-H_2O (4:1:5, v/v/v). The chromatograms were dried and scanned for radioactivity with a Packard radiochromatogram scanner. Specific radioactive peaks were located and identified by co-chromatography with authentic samples and by use of ninhydrin or I_2-NaN_3 sprays. The percent ^{14}C recovered in each peak was estimated by determining the peak area and comparing to the total ^{14}C recovered.

Results and discussion. When 5′-methylthioadenosine-[Me ^{14}C] was fed to post-climacteric apple tissue four radioactive compounds were isolated after a 3 h incubation, with the major metabolites being methionine and 5′-methylthioadenosine (table 1). The incorporation of ^{14}C into methionine from 5′-methylthioadenosine was consistently 90% higher than that reported previously[5]. About 20% of the radioactivity was recovered in 2 unknown metabolites, 1 which remained near the origin (R_f = 0.04) and 1 which migrated near the solvent front. Compound B appears to be a degradation product of 5′-methylthioadenosine. Our interest centered on the unknown near the origin since its migration in this solvent system was similar to that of chemically synthesized methionine-pyridoxal Schiff base, a proposed intermediate between methionine and ethylene[4-6].

In order to determine if the unknown could be an intermediate, methionine-[Me-^{14}C] was fed to the tissue and incubated in air or N_2. 2 inhibitors of ethylene synthesis were also included: L-canaline, an inhibitor of pyridoxal-mediated reactions, and 2,4-dinitrophenol (DNP), a respiratory uncoupler. 3 radioactive compounds were isolated and identified as methionine, 5′-methylthioadenosine and the unknown compound (table 2). In the presence of N_2 or air and 0.1 mM DNP the amounts of unknown and nucleoside relative to air were increased and decreased, respectively. In the presence of air and 0.1 mM canaline the amounts of these products relative to air were decreased in a similar manner. The radioactive spot from R_f 0.04 was identified as a methionine-pyridoxal Schiff base according to the following criteria: on paper electrophoresis at pH 1.9, 6.8, and 10.0 and on paper chromatography, the radioactivity migrated the same distance as chemically synthesized ^{14}C-labelled and unlabelled methionine-pyridoxal Schiff base.

It has been suggested that methionine is first converted to S-adenosylmethionine prior to binding with pyridoxal co-enzyme in ethylene synthesis[4,5] (figure). This is based on the observation that DNP inhibited the formation of labelled ethylene by 80% in apple tissue fed radioactive methionine[4]. If ATP is required in the formation of S-adenosylmethionine prior to binding with pyridoxal co-enzyme, it would be expected that the level of both the Schiff base and 5′-methylthioadenosine (a product of ethylene synthesis) would be decreased by approximately the same amount in the presence of DNP. However, both

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DNP and N_2 greatly increased the level of Schiff base and decreased the level of nucleoside in the tissue. These results suggest that the role of ATP in ethylene synthesis may occur after the formation of a methionine-pyridoxal complex.

Oxygen is required for the conversion of methionine to ethylene, but it has been found that the lag period observed in the conversion of methionine to ethylene is oxygen-independent[10]. This suggests that conversion of methionine into ethylene proceeds in 2 steps: the first step is the oxygen-independent conversion of methionine to an intermediate and the second step is the oxygen-dependent conversion of the intermediate to ethylene. One may expect that the level of the intermediate would increase under a N_2 atmosphere as compared to air. According to the reaction scheme proposed for the conversion of ethylene[4] (figure), the oxygen-independent step would be the conversion of a pyridoxal phosphate enzyme into a pyridoxal-methionine Schiff base. If this is correct, it would be expected that the Schiff base would accumulate under a N_2 atmosphere. Since L-canaline binds stoichiometrically with pyridoxal phosphate[15], it would follow that the formation of pyridoxal-methionine Schiff base would be decreased in the presence of canaline (table 2).

The importance of monitoring the levels of 5'-methylthioadenosine stems from observations that no volatile sulfur compounds are released during ethylene synthesis[8,9], and the conclusion that the methylthio group must be retained and recycled into methionine in order to sustain ethylene production during fruit ripening[9]. In yeast it has been shown that 5'-methylthioadenosine is first converted to methionine prior to formation of S-adenosyl-L-methionine[16]. The present experiments further support the hypothesis that in apple tissue 5'-methylthioadenosine acts as the donor compound in cycling the methylthio group back into methionine[5,7]. As yet it is not known whether this step is a transmethylthiolation or if transfer of the methyl group occurs independent of the sulfur atom. As well, the acceptor compound remains unknown. We are presently investigating this step as it may regulate the rate of ethylene formation since retention of sulfur in the tissue is necessary for continued synthesis of ethylene from methionine.

Caution must be exercised in the interpretation of this data, since methionine can react with other pyridoxal enzymes and the methylthio group may be transferred into other sulfur amino acids as well as methionine which may not be involved in ethylene synthesis. However, it has been reported that conversion of exogenously supplied methionine into ethylene is the major metabolic pathway for methionine in postclimacteric apple tissue, provided the amount of methionine supplied is very small[8].

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Identification of thromboxane B_2 in guinea-pig uterine homogenates[1]

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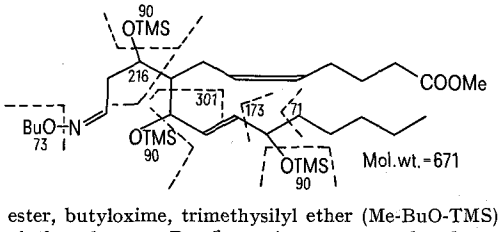
Summary. On the basis of gas chromatographic and mass spectrometric evidence, thromboxane B_2 has been identified in incubates of homogenised guinea-pig uterus.

Homogenates of guinea-pig uterus synthesize prostaglandin $F_{2\alpha}$ (PGF_2) and smaller quantities of prostaglandin E_2 (PGE_2) from endogenous precursors when incubated in vitro[4]. This result has been confirmed using a uterine microsomal enzyme preparation and exogenous precursors[2]. More recently, it has been found that homogenized guinea-pig uteri also synthesise 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$) in vitro[3]. This prostaglandin is the more stable metabolite of prostacyclin (PGI_2). In this study, guinea-pig uterine homogenates have been further investigated to see whether they also produce thromboxanes during incubation.

Material and methods. 9 guinea-pigs in dioestrus (vagina closed) were killed by stunning and incising the neck. Each uterus was removed, homogenised in 15 ml Krebs' solution, and incubated at 37 °C for 90 min, being aerated with 5% carbon dioxide in oxygen. Prostaglandins and any thromboxanes formed were solvent extracted[4]. The 3 samples were pooled into 3 groups of 3 extracts, and further purified by silicic acid colum chromatography[4]. Thromboxane B_2 (TXB_2) is eluted in the 'prostaglandin E fraction' from the column. Consequently, this fraction was converted to the methyl ester, trimethylsilyl ether derivative (Me-TMS), methyl ester, butyloxime, trimethylsilyl ether derivative (Me-BuO-TMS) or the methyl ester, methoxime, trimethylsilyl ether derivative (Me-MO-TMS) for analysis by combined gas chromatography and mass spectrometry (GC-MS) by methods described previously[5].



Methyl ester, butyloxime, trimethysilyl ether (Me-BuO-TMS) derivative of thromboxane B_2. Some fragments produced on mass spectrometry.

Results. The Me-TMS derivative of the post-column extract produced a GC peak at carbon value (C value) 24.6, the reported value for this derivative of TXB_2[6].

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