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(−)-2C-Methyl-D-erythrono-1,4-lactone is formed after application of the terpenoid precursor 1-deoxy-D-xylulose

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Abstract Application of [1,2-^{14}C]1-deoxy-D-xylulose, the committed precursor of terpenoids, thiamine and pyridoxol, to a variety of plant species resulted in the labelling of an unknown metabolite. The isolation and purification of this metabolite from *Ipomoea purpurea* plants fed with 1-deoxy-D-xylulose (DX), followed by NMR analysis, resulted in the identification of its structure as (−)-2C-methyl-D-erythrono-1,4-lactone (MDEL). MDEL has been previously isolated as a stress metabolite of certain plants. A hypothetical biosynthetic scheme is given.

Key words: 1-Deoxy-D-xylulose; (−)-2C-Methyl-D-erythritol; (−)-2C-Methyl-D-erythrono-1,4-lactone; *Ipomoea*; *Liriodendron*; *Tanacetum*

1. Introduction

Application of 1-deoxy-D-xylulose (DX) to several plant species has unequivocally demonstrated this sugar to be the precursor of thiamine [1], pyridoxol [2] and a range of terpenoids ([3] and literature cited therein). Mevalonic acid has been clearly ruled out as the predominant precursor for this type of compounds. Work on the non-mevalonoid terpenoid pathway showed that (−)-2C-methyl-D-erythritol (MDE) is an intermediate, since the deuterium labelled compound was incorporated into menaquinone and ubiquinone when fed to *E. coli* [4]. Subsequently it was shown that DX is transformed into MDE via a pinacol-type skeletal rearrangement [5]. An enzyme that catalyzes the conversion of DX-phosphate to MDE-phosphate in the presence of NADPH has recently been identified [6].

During our investigation of MDE and terpenoid formation, ^{14}C-labelled DX was fed to a range of plants. Two-dimensional TLC of the crude extracts obtained revealed the presence of a new metabolite, which in some cases was a dominant metabolite aside from unmetabolized DX. Feeding of DX at a concentration of 15 mM to an easily accessible plant (*Ipomoea purpurea*) yielded this unknown compound that, after purification, was identified by ^{1}H- and ^{13}C-NMR as (−)-2C-methyl-D-erythrono-1,4-lactone (MDEL). MDEL up to now has been found to occur on three occasions [7–9] in the plant kingdom and is considered to be a stress metabolite associated with water deficiency [8].

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2. Materials and methods

2.1. Plant material and feeding conditions

Plants were grown in a green house. Leaves or shoots with attached leaves weighing about 150 mg (fwt) were dissected from the plants and placed into vials containing [1,2-^{14}C]DX (0.3 μCi; 62.5 mCi/mmol) in 0.5 ml of H_2O. The plant material was incubated at 20 ± 1°C and 50% relative humidity in continuous light. The solution containing the labelled keto-sugar was allowed to be taken up by the plant tissue and water was added when necessary. After 24 h, the tissue was cut into small pieces and extracted with boiling H_2O under reflux.

2.2. Isolation of metabolites

The aqueous extract was concentrated and chromatographed in two dimensions on silica gel G in ethyl acetate/ethanol/glacial acetic acid 84:15:1 (unknown compound R_f 0.39) and in the second dimension using acetone/water/chloroform/methanol 50:5:35:10 (unknown compound R_f 0.44).

For the isolation of amounts of the unknown metabolite sufficient for NMR analysis, 1 mg of unlabelled DX was dissolved in 500 μl H_2O to give a 15 mM solution. One hundred shoots of *Ipomoea purpurea* weighing about 500 mg (fwt) each were placed singly into Eppendorf vials and were allowed to take up the solution containing the pentose over a period of 24 h at 20 ± 1°C and 50% relative humidity. After this period of time, the tissue was extracted with boiling H_2O as given above. The aqueous extracts were concentrated to 100 ml and extracted three times with water saturated *n*-butanol (3 × 100 ml). The organic phase contained, among other compounds, DX, small amounts of MDE and the unknown compound. The butanol phase was taken to dryness, the residue taken up in 10 ml of H_2O and 650 mg of Girard-P reagent (acethydrazide pyridinium chloride) in 2 ml glacial acetic acid was added. The mixture was kept at room temperature for 12 h. Aldoses, ketoses, as well as DX form hydrazones under these conditions while the unknown compound does not react. The unknown compound was subsequently isolated by preparative TLC (silica gel G) in the solvent systems given above resulting in 1.3 mg of the pure compound.

2.3. Spectral analysis

^{1}H- and ^{13}C-NMR spectra were recorded with a Bruker AM 360 spectrometer at 360 MHz and at 90.6 MHz, respectively, in [D_4]methanol as solvent. The optical rotation angle $[\alpha]_D$ was determined on a Perkin-Elmer 241 MC polarimeter. CIMS and EIMS spectra were recorded on a Finnigan MAT SSQ 700 mass spectrometer.

2.4. Materials

All chemicals were purchased from Sigma and Aldrich at the highest purity available. L-[U-^{14}C]Alanine (156 mCi/mmol) was obtained from Amersham-Buchler and converted to pyruvate by action of glutamate pyruvate transaminase (Sigma). [1,2-^{14}C]DX was synthesized in ca. 80% yield from [U-^{14}C]pyruvate and D-glyceraldehyde by catalysis of the pyruvate dehydrogenase complex [10] as reported previously [11]. Pyruvate dehydrogenase was isolated from *E. coli* and used in the formation of [1,2-^{14}C]DX. Authentic natural (−)-2C-methyl-D-erythritol was a kind gift from Prof. Dittrich, Munich, Germany and Prof. Angyal, Kensington, Australia.

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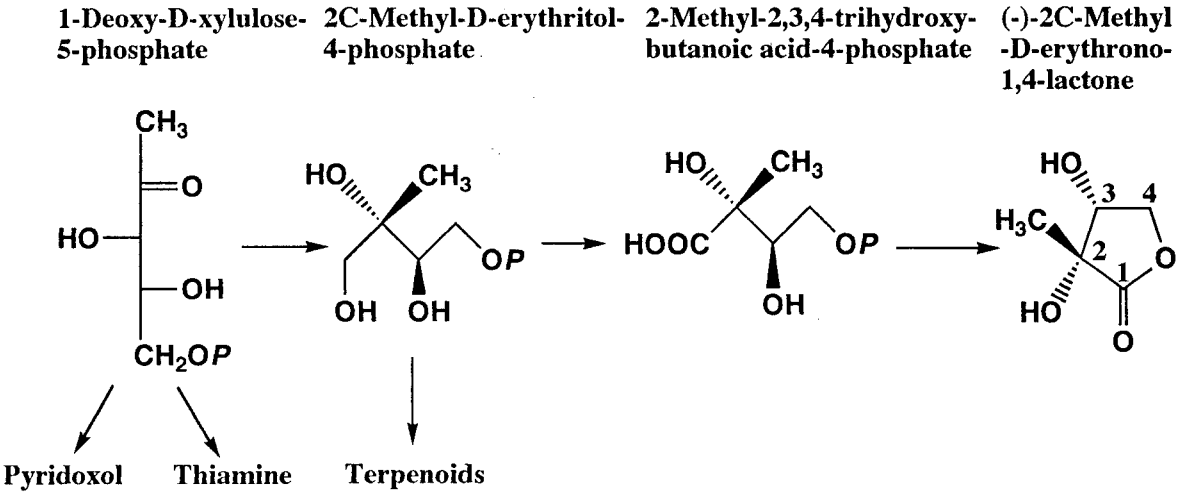

Fig. 1. Putative biosynthetic pathway leading from 1-deoxy-D-xylulose-5-phosphate to (−)-2C-methyl-D-erythrono-1,4-lactone.

3. Results and discussion

In our attempts to characterize intermediates of the DX pathway leading to terpenoids in higher plants, we fed *Liriodendron tulipifera* [12] leaves as well as *Ipomoea parasitica* shoots with ^{14}C-labelled DX [5]. Both species yielded MDE in radioactively labelled form. Using [^{13}C]DX it was clearly shown that MDE is formed from DX via a pinacol-type rearrangement, mechanistically very similar to distinct steps in the formation of riboflavine [13], valine [14] and apiose [15]. Careful examination of the radioactively labelled extracts both of *L. tulipifera* and of *I. parasitica* showed the occurrence of a hitherto unknown metabolite of [1,2-^{14}C]DX. Because of its polarity this compound was neither a phosphate nor a pyrophosphate ester. Upon 2-D-TLC, this metabolite was found between DX and MDE. Feeding of carrier-free labelled DX of high specific activity to the more readily available *Ipomoea purpurea* demonstrated again the formation of this unknown metabolite of DX in about 10% yield; by comparison the conversion of DX to MDE was only about 6%. In order to identify the structure of this unknown compound and to learn whether it is a dephosphorylated intermediate between DX or possibly MDE, it was decided to isolate this metabolite in sufficient quantity for structure elucidation.

Allowing *I. purpurea* shoots to take up an aqueous solution of unlabelled DX spiked with 0.3 μCi ^{14}C-labelled DX, demonstrated a conversion rate of ca. 5% of DX to the unknown compound. The aqueous extract of the shoots was extracted with *n*-butanol which resulted in an enrichment of the unknown compound but also in the contamination of the extract with unmetabolized DX. In order to free the extract from aldoses and ketoses including DX, the aqueous extract was treated with Girard-P reagent. Further purification of the enriched extract by preparative TLC yielded the unknown compound.

NMR spectroscopy of this compound yielded the following data: ^{1}H-NMR: 1.27 ppm (3H, *s*, H-5β), 3.93 ppm (1H, *d*, $J = 4.0$ Hz, H-3β), 4.03 ppm (1H, *d*, $J = 10.4$ Hz, H-4α), 4.32 ppm (1H, *dd*, $J = 10.4$ and 4.0 Hz, H-4β); ^{13}C-NMR: 21.6 ppm (C-5), 73.3 ppm (C-4), 74.6 ppm (C-3), 75.5 ppm (C-2), 180.4 ppm (C-1). Furthermore recording of mass spectra led to CIMS, *m/z* (rel. int.): 133 ($M+H^{+\bullet}$, 100) and EIMS, *m/z* (rel. int.): 132 ($M^{+\bullet}$, 5), 117 (5), 104 (35), 85 (20), 83 (20), 74 (55), 70 (60), 55 (100) confirming the reported data [7]. The optical rotation angle was determined as $[\alpha]_D^{20} = -29.2°$ (0.8, MeOH) in good agreement with the literature [8]. The structure of this compound is therefore (−)-2C-methyl-D-erythrono-1,4-lactone, which is a known compound [7,9]. It is, however, the first time that this compound has been demonstrated to be derived from DX. This lactone has been shown to occur as a natural product in water-stressed chickpea (*Cicer arietinum*) [8], in *Astragalus lusitanicus* [7] and in the toxic plant *Hymenoxys richardsonii* [9]. Feeding of carrier-free [1,2-^{14}C]DX to a number of differentiated plants (Table 1) showed that in each case the lactone was formed in about 10% yield even in plants which did not accumulate MDE. Since all chlorophyll-containing higher plants thus far investigated synthesize phytol and carotenoids from DX, the formation of MDEL indicates that this metabolite is perhaps not a stress metabolite in higher plants, but it could rather be argued that this compound is a normal side product from overshooting DX metabolism.

Table 1
Percent incorporation of [1,2-^{14}C]1-deoxy-D-xylulose into (−)-2C-methyl-D-erythrono-1,4-lactone and (−)-2C-methyl-D-erythritol

Plant species	2C-Methyl-D-erythrono-1,4-lactone (%)	2C-Methyl-D-erythritol (%)
Convolvulus arvensis	10.2	20.6
Convolvulus glomeratus	9.7	9.1
Convolvulus tricolor	9.1	13.7
Ipomoea parasitica	7.9	26.9
Ipomoea purpurea	9.8	5.7
Liriodendron tulipifera	9.3	15.2
Mentha × piperita	8.4	0
Tanacetum vulgare	9.4	0

The potential biosynthetic sequence of this lactone could be envisaged as shown in Fig. 1. The known 1-deoxy-D-xylulose-5-phosphate [16–18] is transformed by the new enzyme 1-deoxy-D-xylulose-5-phosphate reducto isomerase [6] to 2C-methyl-D-erythritol-4-phosphate. This compound is in turn oxidized at the alcoholic group at C-1 to a carboxylic acid by a dehydrogenase. Dephosphorylation yields the free acid which spontaneously forms the lactone. We assume that higher plants have in general the capability to irreversibly metabolize DX by conversion to the erythrono lactone even if supplied in minute amounts (as in the case of the radioactively labelled compound) or if force fed with rather high amounts (15 mM) or when endogenous metabolism directs DX towards an accumulation of the branched erythritol derivative, a key compound in plant terpenoid metabolism.

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