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BIOSYNTHESIS OF 4-HYDROXY-5-METHYLCOUMARIN IN A *GERBERA JAMESONII* HYBRID

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Key Word Index—*Gerbera jamesonii*; Compositae; biosynthesis; 4-hydroxy-5-methylcoumarin.

Abstract—The biosynthesis of 4-hydroxy-5-methylcoumarin was studied by feeding ^{14}C-labelled compounds to *Gerbera jamesonii*. The results reveal that this coumarin is formed via a pentaketide, derived from the acetate–malonate pathway, as in the biosynthesis of 5-methylcoumarin in a fungus.

INTRODUCTION

Gerbera jamesonii hybrid (Compositae) is a familiar garden form developed from *G. jamesonii* Bolus. We have examined the chemical constituents of this *Gerbera* plant and isolated two 5-methylcoumarin glycosides, the glucoside and rutinoside of 4-hydroxy-5-methylcoumarin along with three cyanogenic glycosides, prunasin, amygdalin and vicianin from the underground parts [1].

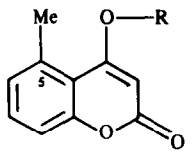
Chexal *et al.* [2] isolated siderin (4,7-dimethoxy-5-methylcoumarin) from *Aspergillus variecolor* (IMI 53749) and reported, after a feeding experiment with ^{14}C-labelled acetate to this fungus, that siderin is formed via a pentaketide. Siderin was also isolated from *Sideritis canariensis* [3] and *S. romana* [4] (Labiatae).

As xanthones can be formed by different biosynthetic routes in fungus and in higher plants, it is of interest to examine whether 5-methylcoumarin in a higher plant is formed via the pathway operating in a fungus. Further investigation of the aerial part of the plant revealed that it contains 4-hydroxy-5-methylcoumarin glucoside in sufficient amount to perform such a biosynthetic study.

RESULTS AND DISCUSSION

The dried aerial parts of the *Gerbera jamesonii* hybrid were extracted with methanol and the methanolic extract was treated with hot water. The chromatography of water-soluble fraction with polyamide afforded 4-hydroxy-5-methylcoumarin glucoside (**1**), which was identified by comparison with an authentic sample.

Various ^{14}C-labelled compounds were fed to *Gerbera jamesonii* by the cotton-wick method. After feeding for three or seven days, 4-hydroxy-5-methylcoumarin glucoside (**1**) was isolated from the aerial parts and then it was hydrolysed with β-glucosidase to yield 4-hydroxy-5-methylcoumarin (**2**). L-[U-^{14}C]Phenylalanine was very poorly incorporated into the coumarin (**2**) but [1-^{14}C]- and [2-^{14}C] acetate, and [2-^{14}C] malonic acid were clearly incorporated into **2**, though the incorporation ratios are relatively low (Table 1). These findings indicate that **2** is not formed via shikimate but unequivocally via the polyketide pathway.



1 R = Glc
2 R = H

Furthermore, radioactive coumarin (**2**) from [1-^{14}C]- and [2-^{14}C]acetate and [2-^{14}C]malonic acid feeding were subjected to Kuhn–Roth oxidation to ascertain the distribution of the label in the starting acetate (methyl group and C_5). The radioactivities of the resulting acetic acid (**3**) were counted as the *p*-bromophenacyl ester.

The ratio of specific activities of acetic acid (**3**) to those of coumarin (**2**) are shown in Table 1. In the case of [1-^{14}C]- and [2-^{14}C]acetic acids feeding, the distribution of label in acetic acid (**3**) is 18.6% of the activity of coumarin (**2**). In the biosynthesis of siderin by [2-^{14}C] acetate feeding, Chexal *et al.* [2] reported that the activity of the starting acetate is 25.8% of that of siderin. Although an even incorporation of acetate into pentaketide-derived compound would be 20%, this ratio appears to vary according to the experimental conditions. These results indicate that coumarin (**2**) arises from five acetate units, as in siderin formation in fungus.

[2-^{14}C]Malonic acid was also incorporated into the acetic acid (**3**) at the rate of *ca* 10%. Bu'Lock *et al.* [5] studied the biosynthesis of 6-methylsalicylic acid in *Penicillium urticae* and demonstrated that [2-^{14}C] malonate can be incorporated into the starting acetate at the rate of 8%, depending on the experimental conditions. These findings can be explained by incorporation of acetyl CoA formed by decarboxylation of malonyl CoA. These results show that the starting acetate unit is formed from acetyl CoA and the other four acetate units from malonyl CoA.

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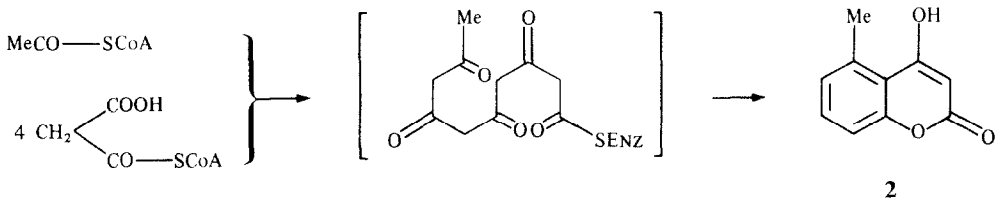
Table 1. Incorporation of labelled compounds into 4-hydroxy-5-methylcoumarin (2) and radioactivities of acetic acid (3) from Kuhn–Roth oxidation

Precursors	Act. (μCi)	Feeding Period (days)	Coumarin (2) Sp. act. (dpm/mM)	Coumarin (2) Incorporation (%)	Coumarin (2) Dilution	Acetic acid (3) Sp. act.* (dpm/mM)	Acetic acid (3) Ratio† (%)
L-[U-^{14}C] Phenylalanine	50	3	5.84×10^2	<0.001			
[2-^{14}C] Sodium acetate	50	3	1.50×10^4	0.0044	7.99×10^6		
[2-^{14}C] Sodium acetate	250	7	2.25×10^5	0.0088	5.82×10^5	4.19×10^4	18.6
[1-^{14}C] Sodium acetate	250	7	1.64×10^5	0.015	7.81×10^5	3.01×10^4	18.6
[2-^{14}C] Malonic acid	250	7	1.10×10^6	0.066	1.03×10^5	1.09×10^5	9.9

*Counted as *p*-bromophenacyl acetate.
†Percentage to the specific activity of coumarin (2).



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Scheme 1. Proposed biosynthetic pathway of 4-hydroxy-5-methylcoumarin in *Gerbera jamesonii*.

This inference is supported by the fact that [1-^{14}C]-malonic acid was better incorporated into coumarin (2) than were [1-^{14}C]- and [2-^{14}C]acetates.

Thus we propose that 4-hydroxy-5-methylcoumarin is biosynthesized via a pentaketide derived from one acetate and four malonates as shown in Scheme 1. Consequently 5-methylcoumarin is formed by the same pathway in higher plants and in fungi.

EXPERIMENTAL

Plant material and radiochemicals. Gerbera jamesonii hybrid was purchased from Sakata-shubyo Co. Ltd (Kanagawa prefecture, Japan). [1-^{14}C]Sodium acetate, [2-^{14}C]sodium acetate and L-[U-^{14}C]phenylalanine were obtained from Amersham International plc; [2-^{14}C] malonic acid from New England Nuclear. The radioactivities of the samples were measured by liquid scintillation counting.

Isolation of 4-hydroxy-5-methylcoumarin glucoside (1). The air-dried aerial parts (100 g) of the above plant were extracted with hot MeOH (500 ml) several times and the MeOH extracts concd under red. pres. The residue was mixed with hot H_2O (500 ml) and the insolubles filtered off. The aq. solution was concd under red. pres. to *ca* one-tenth of the original vol. and chromatographed on polyamide. Elution with H_2O afforded crystals, which were recrystallized from H_2O to give colourless needles (630 mg), mp 152–153°. This compund was identified as 4-hydroxy-5-methylcoumarin glucoside (1) by comparison (mmp, TLC and IR) with an authentic sample.

Feeding experiments. Labelled compounds dissolved in H_2O (0.5 ml) were administered to the base of the radical leaves by the cotton-wick method. A small amount of H_2O was frequently supplied in order to make ensure absorption of the remaining precursors. After 3 or 7 days feeding, the aerial part was cut off and dried (12–13 g).

Hydrolysis of 4-hydroxy-5-methylcoumarin glucoside (1). Radioactive coumarin glucoside was isolated from dried aerial parts (12–13 g) according to the procedure described above. β-Glucosidase (10 mg) was added to the soln of 1 (50 mg) in a mixture of Na_2HPO_4–citric acid buffer (pH 5) and EtOH (2:1) (25 ml). After stirring for 24 hr at 37°, the mixture was concd under red. pres. to give the crystalline product, which was recrystallized from EtOH–H_2O to give 4-hydroxy-5-methylcoumarin (2) as colourless needles (20 mg), mp 233–236°.

Kuhn–Roth oxidation of 4-hydroxy-5-methylcoumarin (2). Coumarin (2) was diluted *ca* 5–10 times with carrier material. The oxidizing solution was prepared by adding H_2SO_4 (25 ml) to the solution of CrO_3 (16.8 g) in H_2O (100 ml) [6]. Coumarin (2) (100 mg) was dissolved in the oxidizing solution (50 ml) and refluxed for 1.5 hr at *ca* 130°. After cooling, dry $MgSO_4$ (7 g) was added to the reaction mixture, which was distilled with occasional addition of an adequate amount of H_2O until 50 ml of distillate was obtained. The distillate was neutralized with 0.1 M NaOH and concd to a small vol. under a N_2 stream and red. pres. *p*-Bromophenacyl bromide (130 mg) and EtOH (3 ml) were added to the above concd soln, and the mixture was refluxed for 30 min. After cooling, the mixture was concd to dryness under red. pres. and chromatographed on silica gel. Elution with C_6H_6 afforded *p*-bromophenacyl acetate, which was recrystallized from hexane (96 mg), mp 84–85°.

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