Phytochemistry, Vol. 26, No. 5, pp. 1409–1411, 1987.
Printed in Great Britain.

0031-9422/87 $3.00 + 0.00
Pergamon Journals Ltd.

BIOSYNTHESIS OF ACEROGENIN A, A DIARYLHEPTANOID FROM *ACER NIKOENSE*

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(*Received* 28 *May* 1986)

Key Word Index—*Acer nikoense*; Aceraceae; biosynthesis; diarylheptanoid; acerogenin A.

Abstract—The biosynthesis of acerogenin A was studied by feeding various ^{14}C-labelled compounds to the young shoots of *Acer nikoense*. Phenylalanine and cinnamic acid were the best precursors. C-2 of both acetate and malonic acid were efficiently incorporated into acerogenin A, but C-1 of acetate and the methyl carbon of methionine were incorporated very poorly. These results show that acerogenin A is probably biosynthesized via (−)-centrolobol derived from two *p*-coumarate residues and one malonate.

INTRODUCTION

Diarylheptanoids have been found in plants of the families Betulaceae [1–5], Zingiberaceae [6–8], Myricaceae [9–11], Leguminosae [12], Aceraceae [13–18], Dioscoreaceae [19] and Bruceraceae [20] as natural products possessing a C_6–C_7–C_6 carbon skeleton. Their structures are classified into linear and cyclic types, and the latter is divided further into biphenyl and diphenyl ether types. Two cyclic types have been postulated to be formed from the corresponding linear types by phenolic oxidative coupling [21]. 9-Phenylphenalenones distributed in the Haemodoraceae are also known as natural products related biosynthetically to diarylheptanoids [22].

Regarding tracer experiments to study the biosynthesis of diarylheptanoids, Roughley and Whiting [23] have demonstrated that curcumin is probably formed from one cinnamate unit and five malonate units in *Curcuma longa* whereas it would be expected to be derived from two cinnamates and one malonate. In contrast, Thomas [24] and Edwards and co-workers [25, 26] have reported that 9-phenylphenalenones, the aglycones of haemocorin and lachnanthoside, are formed from one each of phenylalanine and tyrosine, and the methyl carbon of acetate.

We have isolated several cyclic diarylheptanoids from the stem bark of *Acer nikoense*. These are acerogenin A (**1**) [13, 14] and its glycosides (acerosides I (**2**) [13, 14], III (**3**) [15] and VI (**4**) [15]), acerogenin B [16] and a glucoside of acerogenin C [17] (aceroside IV [17]) together with an arylbutanol, (+)-rhododendrol [13] and its glycosides epirhododendrin [13] and apiosylepirhododendrin [15]. Recently, two glycosides of a linear diarylheptanoid, acerosides VII (**5**) [18] and VIII (**6**) [18], were isolated from the same source and their aglycones were confirmed to be (−)-centrolobol (**7**), which seems to be an intermediate of cyclic diarylheptanoids in *Acer nikoense*.

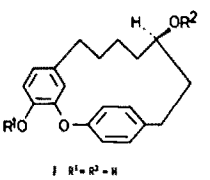
On the other hand, Klischies and Zenk [27] reported that (−)-rhododendrol, the aglycone of rhododendrin, is formed from *p*-coumarate and the methyl carbon of methionine in *Alnus glutinosa* and *Betula alba*. The methyl carbon of methionine is known to provide the C-1 unit in the biosynthesis of some natural products.

We have studied the biosynthesis of acerogenin A (**1**) and now propose that it can be formed via (−)-centrolobol derived from two *p*-coumarate units and one malonate unit.

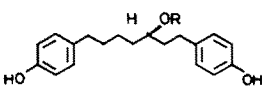
RESULTS AND DISCUSSION

Various labelled compounds were fed to the young shoots of *Acer nikoense*. After feeding for 50 or 70 hr, acerogenin A (**1**) was isolated from the acid hydrolysate of the methanol extract of the bark. Furthermore, acerogenin A (**1**) was degraded by methylation with diazomethane followed by oxidation with potassium permanganate to afford a dicarboxylic acid (3-carboxy-6-methoxyphenyl-4-carboxyphenyl ether) (**8**) by elimination of a five-carbon chain from **1** (Scheme 1).

As shown in Table 1, L-[1-^{14}C]phenylalanine, DL-[3-^{14}C]phenylalanine and [3-^{14}C]cinnamic acid were efficiently incorporated as expected into acerogenin A (**1**). When DL-[3-^{14}C]phenylalanine or [3-^{14}C]cinnamic acid was fed, about 95% of the radioactivity of **1** remained in the dicarboxylic acid (**8**), but in the case of L-[1-^{14}C]phenylalanine feeding only about 5% of the radioactivity of **1** remained in **8**. These facts show intact incorporation of a cinnamate unit into **1**.



1 $R^1=R^2=H$
2 $R^1=Glc$, $R^2=H$
3 $R^1=H$, $R^2=Glc-Api$
4 $R^1=H$, $R^2=Glc$



5 R = Glc
6 R = Glc-Api
7 R = H

Glc = β-D-glucopyranosyl
Glc-Api = β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyl

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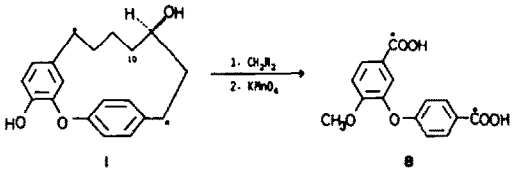
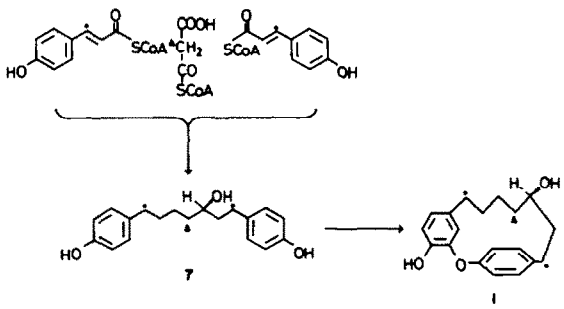
Table 1. Incorporation of labelled compounds into acerogenin A and dicarboxylic acid 8

Experiment	Precursors	Amount fed (μCi)	Acerogenin A Yield* (mg)	Acerogenin A Sp. act. (dpm/mM)	Acerogenin A Incorporation (%)	Dicarboxylic acid 8 Sp. act. (dpm/mM)	Dicarboxylic acid 8 Ratio† (%)
1.	L-[1-^{14}C]Phenylalanine	50	170	6.92×10^4	0.036	3.32×10^3	4.8
2.	DL-[3-^{14}C]Phenylalanine	100	45	7.65×10^5	0.052	7.32×10^5	95.7
3.	[3-^{14}C]Cinnamic acid	100	140	1.56×10^5	0.033	1.47×10^5	94.2
4.	[2-^{14}C]Sodium acetate	250	59	1.08×10^6	0.039	1.98×10^4	1.8
5.	[1-^{14}-C]Sodium acetate	250	95	2.17×10^3	<0.001		
6.	[2-^{14}C]Sodium acetate	250	85	3.81×10^5	0.020		
7.	[2-^{14}C]Malonic acid	100	110	3.24×10^5	0.054	8.90×10^3	2.7
8.	L-[Methyl-^{14}C]methionine	50	50	4.23×10^3	<0.001		

*From dried stem bark (9–32 g).
†Ratio shows percentage to the specific activity of acerogenin A.
Feeding periods, experiments 1–4, 8: 50 hr; experiments 5–7: 70 hr.



Scheme 1. Degradation of acerogenin A.



Scheme 2. Probable biosynthetic route of acerogenin A.

[2-^{14}C]Sodium acetate and [2-^{14}C]malonic acid were efficiently incorporated into acerogenin A (1) but [1-^{14}C]sodium acetate was very poorly incorporated into 1. In addition, the radioactivities of the dicarboxylic acid (8) recovered from [2-^{14}C]sodium acetate and [2-^{14}C]malonic acid feeding were found to be less than 3% of the radioactivity in 1. The above results suggest that the radioactivities of 1 from [2-^{14}C]sodium acetate and [2-^{14}C]malonic acid feedings are present in the central carbon (C-10) of the heptane chain of 1. On the other hand, the poor incorporation of L-[methyl-^{14}C]methionine indicated that the methyl carbon does not seem to be utilized to provide the central carbon (C-10).

Roughley and Whiting [23] reported that [1-^{14}C]- and [2-^{14}C]acetate and [1-^{14}C]- and [2-^{14}C]malonate, as well as [1-^{14}C]- and [3-^{14}C]phenylalanine, were incorporated into curcumin and that the distribution of label showed the participation of both C-1 and C-2 of acetate or malonate in the formation of one aryl ring and four carbons of heptane chain.

Comparing the incorporation results of [1-^{14}C]- and [2-^{14}C]acetate into acerogenin A (1) and curcumin, it is concluded that acerogenin A is formed by a different route from that used for curcumin production. From all the feeding experiments we thus propose that acerogenin A (1) can probably be biosynthesized by cyclization of (−)-centrolobol (7) derived from two *p*-coumarate units and one malonate unit as shown in Scheme 2.

EXPERIMENTAL

Radiochemicals. L-[1-^{14}C]Phenylalanine, [1-^{14}C] sodium acetate, [2-^{14}C]sodium acetate and L-[methyl-^{14}C]methionine were obtained from Amersham International U.K.; DL-[3-^{14}C]phenylalanine and [3-^{14}C]cinnamic acid from Commissariat A l'Energie Atomique; and [2-^{14}C]malonic acid from New England Nuclear. The radioactivities of the samples were measured by liquid scintillation counting.

Feeding procedure and isolation of acerogenin A (1). *Acer nikoense*, cultivated at the Medicinal Plant Garden, Hoshi University, was used for the biosynthetic expts. Young shoots (50–60 cm long) of the plants were cut (in June) and immersed in an aq. soln of labelled compound. After feeding for 50 or 70 hr, bark was stripped from the shoots, dried and cut into small pieces. The bark was extracted repeatedly with hot MeOH and the MeOH extract was refluxed with 5% MeOH–HCl for 4 hr. After cooling, $NaHCO_3$ was added to the reaction mixture to give about pH 3. The mixture was diluted with H_2O, then concentrated under red. pres. in order to remove most of the MeOH and extracted with Et_2O. The Et_2O layers were evaporated and the residue was chromatographed on silica gel with C_6H_6–EtOAc (4:1) as eluant to afford crude crystals, which were recrystallized from C_6H_6 to give acerogenin A (1) as colourless needles, mp 152°.

Degradation of acerogenin A (1). Radioactive acerogenin A (1) was diluted about 4–10 times with carrier material. Acerogenin A (1) was methylated with CH_2N_2 as described previously [14] and 10% NaOH (7.5 ml) and $KMnO_4$ (950 mg) dissolved in H_2O (7.5 ml) were added to a pyridine soln of the methyl ether of 1 (100 mg). The mixture was refluxed for 40 min and an excess of $KMnO_4$ was decomposed with MeOH. After the reaction, the mixture was acidified with dilute H_2SO_4 and $NaHSO_3$ was added. The resulting ppt. was filtered and washed with

MeOH–Et_2O. The filtrate and washings were combined and concentrated under red. pres. H_2O was added to the residue and the mixture was extracted with Et_2O. The Et_2O soln was evaporated and the residue was chromatographed on silica gel with $CHCl_3$–MeOH–H_2O (200:55:7) as eluant to obtain dicarboxylic acid **8**, crystallized from EtOH–H_2O to give colourless crystals, mp 298° (20 mg), which was identical with an authentic sample previously obtained by oxidation of acerogenin A methyl ether ketone [14].

Acknowledgement—This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, which is gratefully acknowledged.

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