Natural Occurrence and Biosynthesis of a Cyclopentanoid Monoterpene Carboxylic Acid

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THE postulated intermediacy of cyclopentanoid monoterpenes in the biosynthesis of the related monoterpene glucoside gentiopicroside and other seco-iridoids[1,2] is analogous to the transformation of loganin to the C_{10} moiety of the indole alkaloids.[3]

Further studies with *Swertia caroliniensis* (Gentianacea) in our laboratory has led to the discovery of the co-occurrence of loganic acid with gentiopicroside in this plant.

The acidic fraction of the methanol-soluble extract of fresh *Swertia* plants contained an amorphous glucoside which could be partially purified on a silica-gel column eluted with chloroform–methanol (1 : 1). Further silica-gel chromatographic separation of the acetylated glucoside with chloroform–benzene–methanol (4·5 : 4·5 : 1) afforded a crystalline compound, m.p. 168°, which exhibited characteristic iridoid chromophore absorption in the u.v. region: λ_{max} (EtOH)

230 mμ (log ε 4·2, hypsochromic shift in base) and n.m.r.: δ 7·45 (singlet). The n.m.r. signal at δ 1·05 (doublet, *J* 7 c./sec.) gave the first evidence of a methyl cyclopentanoid structure. In addition, i.r.-spectral data [λ_{max} ($CHCl_3$) 2·87, 5·7, 5·9(sh), 6·11, 7·98 μ] as well as elemental analysis were in agreement with reported values for loganic acid penta-acetate.[4–7] Treatment of the glucoside and its acetylated derivative with diazomethane gave loganin and loganin penta-acetate, respectively. This was demonstrated by identical chromatographic and spectral properties[4–7] as well as an absence of a depression in mixed m.p.s. with authentic samples of loganin and loganin penta-acetate.† Since loganic acid was previously only known as a alkaline hydrolysate of loganin, this represents the first report of its natural occurrence. Its presence with gentiopicroside suggests a metabolic relationship between the two compounds which we further substantiated by the following tracer studies.

TABLE 1

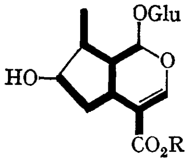
Utilization of [2-^{14}C]*mevalonate in* Swertia caroliniensis

(*Dose*: **25** μc *per experiment*)

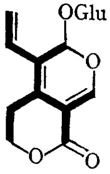
	Monoterpene Glycoside		Weight (mmole)	Specific activity (μc/mmole)	% ^{14}C Converted	Dilution
Expt. 1.	Gentiopicroside	..	0·38	0·02	0·03	1·6 × 10^5
	Loganic acid ..	..	0·03	0·6	0·09	8·3 × 10^3
Expt. 2.	Gentiopicroside	..	0·15	0·025	0·02	2 × 10^5
	Loganic acid ..	..	0·01	4·9	0·3	1 × 10^3
Expt. 3.	Gentiopicroside	..	0·09	0·11	0·04	4·4 × 10^4
	Loganic acid ..	..	0·01	1·4	0·08	3·4 × 10^3

Since the mevalonoid origin of loganin had not previously been established,[3] [2-^{14}C]mevalonate was administered to intact *Swertia* plants by the cotton wick technique.[1] Loganic acid and gentiopicroside were recovered as crystalline polyacetate derivatives. Addition of carrier and recrystallization to constant specific activity provided the counting data compiled in Table 1. In all cases the extent of incorporation of mevalonate into loganic acid was greater than into gentiopicroside, which is consistent with a precursor–product relationship between the two metabolites. Using the same cotton wick feeding technique, previous experiments with *Swertia caroliniensis* have demonstrated the direct incorporation of [2-^{14}C]-mevalonate into the aglucone of gentiopicroside, and labelling in accord with the previously suggested pathway.[1]

In another experiment [1-^{14}C]geranyl pyrophosphate‡ was similarly fed to *Swertia caroliniensis* and, as demonstrated in Table 2, converted into loganic acid. Whereas the fraction of total radioactivity incorporated was diminished, the dilution factor (specific activity of precursor/specific activity of product) which compensates for the relatively low precursor specific activity is comparable to the values obtained in the mevalonate experiment. Recent studies with loganin[3] as well as other cyclopentanoid monoterpenes[8] have also implicated geranyl pyrophosphate in the biogenetic scheme.



Loganic acid, R=H
Loganin, R=Me



Gentiopicroside

TABLE 2

Incorporation of [1-^{14}C]*geranyl pyrophosphate into loganic acid in* Swertia caroliniensis

	[1-^{14}C]-Geranyl PP administered	Loganic acid isolated
Wt. (mg.)	10	2
Specific activity (μc/mmole)	470	0·04
Dose (μc)	10	
% incorporation		0·002
Dilution		1·2 × 10^4

Radioactive gentiopicroside was not obtained in the [1-^{14}C]geranyl pyrophosphate experiment.

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† Kindly provided by Prof. J. Wolinsky, Purdue University.

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The absence of label may be attributed to dilution of the isotopic carbon-containing species with that of the relatively large gentiopicroside pool (54 mg.) in the particular plant used in the experiment. In the case of [2-^{14}C]mevalonate utilization in monoterpene glucoside synthesis (Table 1), the amount of isotopic carbon introduced into loganic acid was at least twice as great as that incorporated into gentiopicroside. A specific activity of gentiopicroside calculated on the basis of this ratio for the [1-^{14}C]geranyl pyrophosphate experiment would be only a few d.p.m./mg.

Structural similarities of loganic acid and gentiopicroside, a common mevalonoid origin in the same plant, as well as analogous conversion of loganin into the C_{10} segment of the indole alkaloids, all suggest a pathway wherein cleavage of the cyclopentanoid ring of loganic acid, dehydrogenation and lactonization would afford gentiopicroside.

Furthermore, the occurrence of loganic acid raises the possibility of a more significant metabolic role of the cyclopentanoid monoterpene carboxylic acids in higher plants.

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[1] C. J. Coscia and R. Guarnaccia, *J. Amer. Chem. Soc.*, 1967, **89**, 1280.
[2] H. Inouye, S. Ueda, and Y. Nakamura, *Tetrahedron Letters*, 1967, 3221.
[3] A. R. Battersby, R. T. Brown, R. S. Kapil, J. A. Martin, and A. O. Plunkett, *Chem. Comm.*, 1966, 890. See accompanying communications of Professors Arigoni and Battersby.
[4] K. W. Merz and H. Lehmann, *Arch. Pharm.*, 1957, **290**, 543.
[5] A. J. Birch and J. Grimshaw, *J. Chem. Soc.*, 1961, 1407.
[6] K. Sheth, E. Ramstad, and J. Wolinsky, *Tetrahedron Letters*, 1961, 394.
[7] S. Bader, C. J. Coscia, and D. Arigoni, unpublished observations.
[8] H. Auda, G. R. Waller, and E. J. Eisenbraun, *J. Biol. Chem.*, 1967, **242**, 4157.