THE BIOSYNTHESIS OF APIOSE IN PARSLEY (APIUM PETROSELINUM L.)

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The branched-chain sugar Apiose first discovered in parsley (Vongerichten, 1901) seems to have a rather widespread occurrence in higher plants (Bell, 1962. Duff and Knight, 1963. Bacon, 1963. Williams and Jones, 1964).

We have investigated the biosynthesis of apiose in parsley, where it occurs as a glycosidic component of apiin (Hemming and Ollis, 1953) and as the corresponding luteolin derivative (Nordstroem et al., 1953).

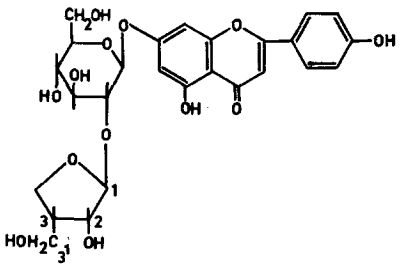


Fig. 1. Apiin. The configuration at C-3 of apiose is not known.

The radioactive precursors were fed to young parsley shoots during 36 hours. Apiin was isolated as the lead salt (Gupta and Seshadri, 1952) and purified by paper chromatography with butanol/ethanol/H_2O (5:1:4) and recristallization from ethanol/H_2O. Hydrolysis with 0.025 N H_2SO_4 on the steam bath yielded apigenin-7-glucoside and

apiose. The latter was converted into its trimethylsilyl-ether (Sweeley et al., 1963) and purified by gas chromatography (16% silicon-gum-rubber on chromosorb W). Apigenin-ß-glucoside was hydrolyzed with 10% H_2SO_4 and the glucose purified by gas chromatography in the same manner. The apigenin was recrystallized as the triacetate from ethanol.

The distribution of activity between apigenin, apiose and glucose and the dilution of activity with different precursors is shown in Table I.

Table I

Incorporation and Distribution of Radioactivity in Apiin with various Precursors

Precursor	Compound	Activity cpm per mmole	Percent activity of apiin	Dilution[a]
Acetate-1-^{14}C 31 µmole + Glucose 300 µmole	Apiin	63300		
	Apiose	12300	19	$6 \cdot 10^7$
	Glucose	4500	7	$15 \cdot 10^7$
	Apigenin	49460	78	
Formate-^{14}C 8.2 µmole + Glucose 82 µmole	Apiin	5650		
	Apiose	3300	58	$3 \cdot 10^6$
	Glucose	1500	27	$6 \cdot 10^6$
	Apigenin	750	13	
Glucose-U-^{14}C 68 µmole + Na-acetate 680 µmole	Apiin	10400		
	Apiose	2850	27	$7 \cdot 10^4$
	Glucose	2830	27	$7 \cdot 10^4$
	Apigenin	5100	49	
Glucose-3.4-^{14}C 13 µmole + Na-acetate 130 µmole	Apiin	110500		
	Apiose	35300	32	$5 \cdot 10^2$
	Glucose	32000	29	$6 \cdot 10^2$
	Apigenin	39500	36	

[a] $\frac{\text{specific activity of precursor}}{\text{specific activity of product}}$

With glucose-U-^{14}C and -3.4-^{14}C (Fiedler and Trebst, 1964) as precursors the specific activities of apiose and glucose are about equal even though inactive acetate was present, and the dilution values are less by a factor of 10^2 to 10^4 as compared with the acetate-1-^{14}C and formate^{14}C experiments. The percentage of activity in apiose is relatively high with formate as precursor but the incorporation of formate into glucose shows that extensive randomization of activity occurred in this experiment. These results make the participation of either acetate (Patrick, 1957) or C_1-units in the biosynthesis of apiose seem unlikely. The equal distribution of activity between apiose and glucose with glucose as precursor, even in the presence of a ten fold excess of unlabeled acetate, led us to the assumption that all 5 carbon atoms of apiose originate from the hexose chain of glucose with loss of one carbon atom.

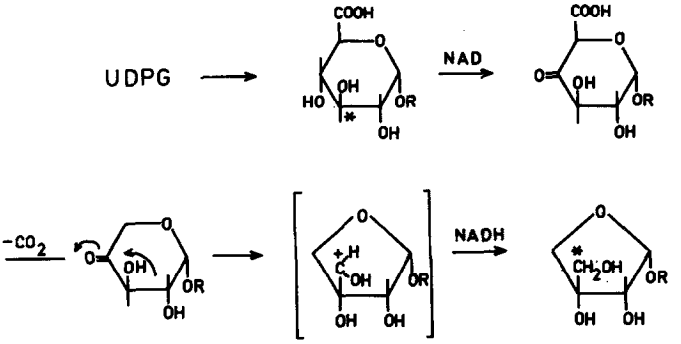
Further insight into the biosynthesis of apiose was gained by oxidation of apiin with periodate in neutral solution. Under these conditions C-3^1 of apiose is converted to formaldehyde which was isolated and purified as the dimedon derivative. As can be seen from Table II C-3^1 contains 23% of the activity of apiose with glucose-U-^{14}C as precursor (20% would be expected for an even distribution of activity in the sugar) and 40% with glucose-3.4-^{14}C as precursor. Carbon 3^1 of apiose must therefore originate from either C-3 or C-4 of glucose.

Table II

Precursor	Compound	Activity cpm per mmole	Percent Activity of Apiose
Glucose-U-^{14}C	Apiose	32000	
	Formaldehyde[a]	7500	23
Glucose-3.4-^{14}C	Apiose	35300	
	Formaldehyde[a]	14000	40

[a] as dimedon derivative

These results can be rationalized by postulating the following sequence of reactions for the biosynthesis of apiose.



The formation of UDP-D-Xylose from UDP-D-Glucuronic acid is known (Bernfeld, 1963) and the observed requirement for NAD led to the hypothesis that the decarboxylation of UDPGA involves the transient formation of a carbonyl intermediate by oxido-reduction at C-4 (Ankel and Feingold, 1964). Such a keto-sugar intermediate could undergo a rearrangement of the type that has been postulated in the biosynthesis of streptose (Candy et al., 1964). Further work to test this hypothesis is in progress.

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REFERENCES

Ankel, H., and Feingold, D.S., Abstr. Sixth Intern. Congress Biochem., New York 1964, Vol VI, p. 502.
Bacon, J.S.D., Proc.Biochem.Soc. 1963, 103 P.
Bell, D.J., In: "Methods in Carbohydrate Chemistry" Vol 1, p. 260. New York: Academic Press, 1962.
Bernfeld, P., In: "Biogenesis of Natural Compounds", p. 305. Oxford: Pergamon Press, 1963.
Candy, D.J., Blumson, N.L., and Baddiley, J., Biochem.J. 91, 31 (1964).
Duff, R.B., and Knight, A.H., Proc.Biochem.Soc. 1963, 33 P.
Fiedler, F., and Trebst, A., Z.Naturforsch. 19b, 395 (1964).
Gupta, S.R., and Seshadri, T.R., Proc.Indian Acad.Science 35, 242 (1952). C.A. 47, 3306c (1953).
Hemming, R., and Ollis, W.D., Chem.and Ind. (London) 1953, 85.
Nordstroem, C.G., and Swain, T., Chem. and Ind. (London) 1953, 85.
Patrick, A.D., Nature 178, 216 (1957).
Sweeley, C.C., Bentley, R., Makita, M., and Wells, W.W., J.Am.Chem.Soc. 85, 2497 (1963).
Vongerichten, E., Ann.Chem. 318, 121 (1901).
Williams, D.T., and Jones, J.K.N., Can.J.Chem. 42, 69 (1964).